

Printed by EAST

UserID: ETHERKORN

5 268 097

Computer: WS08186

Date: 08/03/2000

Time: 09:41

Document Listing

Document	Image pages	Text pages	Error pages
US 6063283 A	0	1	0
US 6054047 A	0	2	0
US 6045697 A	0	4	0
US 5906734 A	0	4	0
US 5906747 A	0	29	0
US 5738783 A	0	1	0
US 5672276 A	0	4	0
US 5599453 A	0	4	0
US 5593576 A	0	4	0
US 5545317 A	0	3	0
US 5512169 A	0	2	0
US 5470463 A	0	4	0
US 5445732 A	0	4	0
US RE34910 E	0	2	0
US 5393430 A	0	4	0
US 5346619 A	0	2	0
US 5271833 A	0	2	0
US 5270280 A	0	2	0
US 5268097 A	0	4	0
US 5182016 A	0	2	0
US 5135649 A	0	1	0
US 5108597 A	0	2	0
US 4314823 A	0	2	0
Total	0	89	0

\~15~

DOCUMENT-IDENTIFIER: US 6063283 A

TITLE: Method for analyzing a sample by using a liquid chromatograph

DEPR:

In the present invention, porous media commonly used in liquid chromatography

such as silica gel, alumina, porous glass beads, zeolite, hydroxyapatite and

graphite may be used as the packing material of the column 40. Alternatively,

it is possible to use composite powders formed of a resin core covered by

inorganic powders such as silica gel, titanium dioxide, hydroxyapatite, and the

like. One may use materials such as polyamides, acrylic resins, polyvinyl

alcohols, and the like, for the resin core.

CCXR:

210/198.2

DOCUMENT-IDENTIFIER: US 6054047 A

TITLE: Apparatus for screening compound libraries

BSPR:

Preferably, the solid phase support used in this invention is selected from the group consisting of resin beads, glass beads, silica chips, silica capillaries and agarose.

DEPR:

When employing the apparatus of this invention, the target receptor is optionally bound or coupled to a solid support. Preferably, the target receptor is covalently bound or coupled to the solid support. However, in some cases, such as when whole cells or organisms are employed as the target receptor, the cells or organisms may be contained within the column by using, for example, a porous frit at the outflow end of the column. Supports for receptors are well-known in the art and many are commercially available. Any such conventional support may be used in this invention. Representative supports include, by way of illustration, resin beads, glass beads, silica chips and capillaries, agarose, and the like. When silica capillaries are used as the solid support, the target receptor is bound directly to the walls of the column. Preferred solid supports for use in this invention include porous resin beads. A particularly preferred solid support is porous polystyrene-divinylbenzene polymer beads, such as POROS beads (available from Perseptive Biosystems, Framingham, Mass.).

DEPR:

When employing multiple columns, each column is typically monitored for a brief period of time before switching to the next column. For example, with a quadrupole mass spectrometer, each column is typically monitored sequentially for a period of about 0.5 seconds to about 10 seconds, preferably

for about 1
second to about 5 seconds, before switching to the next column.
The effluent
from each column is analyzed as described herein using mass
spectrometry.
Generally, a single running file is used to collect all of the
data from the
multiple column thereby generating a composite total ion
chromatogram.
Subsequently, separate total ion chromatograms for each column
are recreated by
synchronizing column switching with mass spectrometry data
acquisition.

CLPR:

16. The apparatus of claim 14, wherein the solid phase support
is selected
from the group consisting of resin beads, glass beads, silica
chips, silica
capillaries and agarose.

CCOR:

210/198.2

DOCUMENT-IDENTIFIER: US 6045697 A

TITLE: Passivated porous polymer supports and methods for the preparation and use of same

BSPR:

Several previous investigators have sought to passivate various microporous media including membranes and particulate chromatographic supports by applying thin surface coatings to inorganic or organic/polymeric substrates. For example, Steuck, in U.S. Pat. No. 4,618,533, discloses a porous polymeric membrane substrate fashioned from a thermoplastic organic polymer upon which a permanent coating is grafted and/or deposited on the entire membrane surface. The polymerization and crosslinking of the polymerizable monomer upon and within the porous membrane substrate is performed in such a way that a thin coating is deposited upon the entire surface of the porous membrane, including the inner pore walls. Significantly, the porous configurations of the coated, composite membrane structures claimed by Steuck are essentially identical to those of the corresponding uncoated porous membrane substrates, implying that the polymer of Steuck is applied as a thin surface layer or coating that does not interfere with the porosity or flow properties of his composite membranes. Moreover, Steuck does not disclose the concept of a "passivating layer" or the use of monomers capable of functioning as "passivating" monomers within the meaning of the present invention as discussed in more detail below.

BSPR:

While it has been discovered that this process of depositing protective polymer coatings upon the porous surfaces of mineral oxide (and particularly silica) matrices can significantly stabilize these materials by sharply reducing their

rates of chemical leaching, the approach has the important disadvantage of rendering the porous surfaces of the coated and protected matrices hydrophobic and thus prone to cause excessive non-specific binding of proteins by adsorption. (This is precisely the same problem noted above in connection with entirely polymeric porous support matrices.) However, this problem can be successfully addressed by the methods of the present invention in the same way as the non-specific binding of strictly polymeric support matrices can be reduced--i.e., by passivation in a process of oriented polymerization. More particularly these composite chromatographic supports (i.e., supports comprised of mineral oxide substrates that have been stabilized by the application of thin protective polymer coatings) can be passivated against excessive non-specific binding by incorporating passivating ("neutralizing") monomers capable of associating with and consequently deactivating innate nonpolar hydrophobic groups exposed on the matrix surface. The passivating monomers useful in this embodiment of the present invention adsorb upon (and consequently cover) the hydrophobic groups on the surface by virtue of their containing long-chain saturated hydrocarbons, olefinic hydrocarbon groups, aromatic groups, or like hydrophobic domains that interact with and become appreciably bound to their hydrophobic counterparts on the matrix surface as a consequence of the hydrophobic-hydrophobic interaction existing between them. Typically, the present invention utilizes base matrices having the following characteristics: an initial average particle size ranging from about 5 to about 1000 μm ; an initial porous volume ranging from about 0.2 to about 2 cm^3/gram ; an initial surface area ranging from about 1 to about 800 m^2/gram ; and an initial pore size ranging from about 50 to about 6000 angstroms.

Preferably, the base matrix is characterized by: an initial average particle size ranging from about 10 to about 300 μm , although passivated supports having narrow particle size ranges, such as about 15-20 μm , about 15-25 μm , about 30-45 μm , about 50-60 μm , about 80-100 μm , and about 100-300 μm , are most preferred. Preferred ranges for other characteristics include an initial porous volume ranging from about 0.8 to about 1.2 cm^3/gram ; an initial surface area ranging from about 10 to about 400 m^2/gram ; and an initial pore size ranging from about 1000 to about 3000 angstroms. The density of the porous solid matrix obviously varies with its chemical nature, being higher for mineral oxide (e.g., silica) substrates and lower for polymeric ones (e.g., polystyrene).

DEPR:

It is thus demonstrated that the level of non-specific adsorption for lysozyme

(a strong cationic protein) is high when the MAPTAC is absent. The

non-specific adsorption for silica with large surface areas (X 075, 100 m^2/g)

is higher (55 mg/mL of resin) than the non-specific adsorption for silica X

015 (25 m^2/g ; 15 mg/mL of resin). A certain proportionality exists

between the surface area and the original level of non-specific absorptions.

The amount of MAPTAC to decrease the level of non-specific adsorption down to

zero is also proportional to the surface area available: 1.5% of MAPTAC is

necessary with silica X 015 (25 m^2/g) whereas at least 6% is necessary to

passivate silica X 075 (100 m^2/g).

DEPR:

The passivated resin is then recovered by filtration. The oil is eliminated

with an extensive washing with water containing 0.1-0.5% of a non-ionic

detergent and then stored in a saline buffer at neutral pH. The product resin

shows very similar ion-exchange characteristics as those described in Example

2. Additionally, its sensitivity in strong alkaline media is much improved as measured by its weight loss after one night of contact with 0.5 M sodium hydroxide. The passivated resin of this example lost only about half as much weight as an anionic resin prepared from silica having an unprotected surface area.

DEPR:

The polymer-silica composite was then washed several times with water, with diluted hydrochloric acid and with diluted sodium hydroxide solution (0.2M).

After neutralization the ion exchanger was used for protein separation. The

number of anionic groups per mL of wet materials was 110 .mu.eq and sorption capacity for cationic proteins (e.g., lysozyme) was 120 mg/mL of resin.

DEPC:

Preparation of an Anion-Exchange Resin Using a Surface-Protected (i.e., Pre-coated) Silica Passivated Porous Support

DEPC:

Determination of Ion-Exchange and Protein Sorption Capacity of Preparation of

Anion-Exchange Resins Based on Passivated Porous Silica Support of Different Surface Areas

CCXR:

210/198.2

DOCUMENT-IDENTIFIER: US 5906734 A

TITLE: Passivated porous polymer supports and methods for the preparation and use of same

BSPR:

Several previous investigators have sought to passivate various microporous media including membranes and particulate chromatographic supports by applying thin surface coatings to inorganic or organic/polymeric substrates. For example, Steuck, in U.S. Pat. No. 4,618,533, discloses a porous polymeric membrane substrate fashioned from a thermoplastic organic polymer upon which a permanent coating is grafted and/or deposited on the entire membrane surface. The polymerization and crosslinking of the polymerizable monomer upon and within the porous membrane substrate is performed in such a way that a thin coating is deposited upon the entire surface of the porous membrane, including the inner pore walls. Significantly, the porous configurations of the coated, composite membrane structures claimed by Steuck are essentially identical to those of the corresponding uncoated porous membrane substrates, implying that the polymer of Steuck is applied as a thin surface layer or coating that does not interfere with the porosity or flow properties of his composite membranes. Moreover, Steuck does not disclose the concept of a "passivating layer" or the use of monomers capable of functioning as "passivating" monomers within the meaning of the present invention as discussed in more detail below.

BSPR:

While it has been discovered that this process of depositing protective polymer coatings upon the porous surfaces of mineral oxide (and particularly silica) matrices can significantly stabilize these materials by sharply reducing their

rates of chemical leaching, the approach has the important disadvantage of rendering the porous surfaces of the coated and protected matrices hydrophobic and thus prone to cause excessive non-specific binding of proteins by adsorption. (This is precisely the same problem noted above in connection with entirely polymeric porous support matrices.) However, this problem can be successfully addressed by the methods of the present invention in the same way as the non-specific binding of strictly polymeric support matrices can be reduced--i.e., by passivation in a process of oriented polymerization. More particularly, these composite chromatographic supports (i.e., supports comprised of mineral oxide substrates that have been stabilized by the application of thin protective polymer coatings) can be passivated against excessive non-specific binding by incorporating passivating ("neutralizing") monomers capable of associating with and consequently deactivating innate non-polar hydrophobic groups exposed on the matrix surface. The passivating monomers useful in this embodiment of the present invention adsorb upon (and consequently cover) the hydrophobic groups on the surface by virtue of their containing long-chain saturated hydrocarbons, olefinic hydrocarbon groups, aromatic groups, or like hydrophobic domains that interact with and become appreciably bound to their hydrophobic counterparts on the matrix surface as a consequence of the hydrophobic-hydrophobic interaction existing between them. Typically, the present invention utilizes base matrices having the following characteristics: an initial average particle size ranging from about 5 to about 1000 microns; an initial porous volume ranging from about 0.2 to about 2 cm.³/gram; an initial surface area ranging from about 1 to about 800 m.²/gram; and an initial pore size ranging from about 50 to about 6000

angstroms. Preferably, the base matrix is characterized by: an initial average particle size ranging from about 10 to about 300 microns, although passivated supports having narrow particle size ranges, such as about 15-20, about 15-25, about 30-45, about 50-60, about 80-100, and about 100-300 microns, are most preferred. Preferred ranges for other characteristics include an initial porous volume ranging from about 0.8 to about 1.2 cm.³ /gram; an initial surface area ranging from about 10 to about 400 m.² /gram; and an initial pore size ranging from about 1000 to about 3000 angstroms. The density of the porous solid matrix obviously varies with its chemical nature, being higher for mineral oxide (e.g., silica) substrates and lower for polymeric ones (e.g., polystyrene).

DEPR:

The passivated resin is then recovered by filtration. The oil is eliminated with an extensive washing with water containing 0.1-0.5% of a non-ionic detergent and then stored in a saline buffer at neutral pH. The product resin shows very similar ion-exchange characteristics as those described in Example 2. Additionally, its sensitivity in strong alkaline media is much improved as measured by its weight loss after one night of contact with 0.5 M sodium hydroxide. The passivated resin of this example lost only about half as much weight as an anionic resin prepared from silica having an unprotected surface area.

DEPR:

The polymer-silica composite was then washed several times with water, with diluted hydrochloric acid and with diluted sodium hydroxide solution (0.2 M). After neutralization the ion exchanger was used for protein separation. The number of anionic groups per ml of wet materials was 110 .mu.eq and sorption

capacity for cationic proteins (e.g., lysozyme) was 120 mg/ml of resin.

DEPC:

Preparation of an Anion-Exchange Resin Using a Surface-Protected (i.e.,

Precoated) Silica Passivated Porous Support

DEPC:

Determination of Ion-Exchange and Protein Sorption Capacity of Preparation of

Anion-Exchange Resins Based on Passivated Porous Silica Support of Different Surface Areas

DEPC:

Preparation of a Cation-Exchange Resin Using a Surface--Protected (i.e.,

Precoated) Porous Silica Support

CCOR:

210/198.2

DOCUMENT-IDENTIFIER: US 5906747 A

TITLE: Separation of molecules from dilute solutions using composite chromatography media having high dynamic sorptive capacity at high flow rates

TTL:

Separation of molecules from dilute solutions using composite chromatography media having high dynamic sorptive capacity at high flow rates

BSPR:

This invention relates to chromatography media and their use. In particular, composite media are disclosed which are characterized by a reversible high sorptive capacity. These media may be passivated to prevent non-specific adsorption of or interaction with biomolecules such as proteins, oligopeptides, polysaccharides, and nucleotides. The composite media of the present invention exhibit characteristics that are highly desirable in chromatographic applications, such as high porosity, physical rigidity, high charge density, and chemical stability under a variety of extreme conditions, and may be used advantageously with especially dilute feed streams in high-flow, high-efficiency mass transfer chromatographic techniques that can be carried out in a fluidized-bed, packed-bed, or other mode of operation.

BSPR:

Several previous investigators have sought to passivate various microporous media including membranes and particulate chromatographic sorbents by applying thin surface coatings to inorganic or organic/polymeric substrates. For example, Steuck, in U.S. Pat. No. 4,618,533, discloses a porous polymeric membrane substrate fashioned from a thermoplastic organic polymer upon which a permanent coating is grafted and/or deposited on the entire membrane surface. The polymerization and crosslinking of the polymerizable monomer upon and

within the porous membrane substrate is performed in such a way that a thin coating is deposited upon the entire surface of the porous membrane, including the inner pore walls. Significantly, the porous configurations of the coated, composite membrane structures claimed by Steuck are essentially identical to those of the corresponding uncoated porous membrane substrates, implying that the polymer of Steuck is applied as a thin surface layer or coating that does not interfere with the porosity or flow properties of his composite membranes. Moreover, Steuck does not disclose the concept of a "passivating layer" or the use of monomers capable of functioning as "passivating" monomers within the meaning of the present invention as discussed in more detail below.

BSPR:

The present invention provides a method for the separation of biological molecules by chromatography. The method of the invention comprises the steps of passing a sample containing a mixture of biological macromolecules including a biological macromolecule of interest through a column packed with a composite media and recovering the biological macromolecule of interest from the sample. The composite media which characterizes the present invention provides a larger dynamic capacity for a biological macromolecule at low initial feed concentrations, for example, those less than about 2 milligrams per milliliter, than the dynamic capacity provided by the same media for the same macromolecule at higher concentrations, for example, those higher than about 2 milligrams per milliliter. The media preferably provides a larger dynamic capacity for said macromolecule at initial feed concentration in the range of about 10 micrograms per milliliter to about 2 milligrams per milliliter than the dynamic capacity provided by the same media for the same macromolecule at initial feed

concentrations in the range of about 2 milligrams per milliliter to about 100 milligrams per milliliter. In a preferred embodiment of the present invention, the sample to be subjected to the separation procedure may have an initial concentration of the biological macromolecule of interest of less than about 2 milligrams per milliliter.

BSPR:

In accordance with the present invention, the media may be a composite media that comprises a porous support comprising voids containing a polymeric network, wherein the composite media provides a value of the flux enhancement factor E greater than about 3, preferably greater than about 20, as determined by the equation $E = \frac{D_{sub.s}}{D_{sub.f}}$ in which $D_{sub.s}$ is the experimentally measured effective intraparticle diffusivity of a molecule of interest, $q_{sub.0}$ is the equilibrium concentration of said molecule within the media particles at equilibrium with $C_{sub.0}$, $D_{sub.f}$ is the diffusivity in free solution of said molecule, $\epsilon_{sub.p}$ is the fractional void volume of the porous support of the composite media, τ is the tortuosity of the porous support of the composite media, and $C_{sub.0}$ is the concentration of said molecule in the feed solution, and is preferably at least about 1 microgram per milliliter, more preferably at least about 10 micrograms per milliliter.

BSPR:

The composite media may be selected to be a chromatographic media, more preferably, ion-exchange chromatography media, so that said biological macromolecules can be separated by (ion-exchange) chromatography.

The chromatography media is preferably one that provides an intraparticle diffusional flux that is faster, for instance at least about 30% faster, than the diffusional flux of the biological macromolecules in solution.

DEPR:

The present invention provides a composite media for the separation of biological molecules by chromatography, such as ion-exchange chromatography.

As noted above, the media provides a flux enhancement factor E of greater than

3 wherein ##EQU2## In a further refinement, the media provides a value for a

second figure of merit or flux enhancement factor E^* of greater than 3, wherein

##EQU3## in which $D_{sub.s}$ is the effective intraparticle diffusivity of the

biological molecule of interest, τ is the tortuosity factor of the

composite media, $D_{sub.f}$ is the diffusivity in free solution of the biological

molecule, and $\epsilon_{sub.p}$ * is the species-dependent inclusion porosity of

the composite media (Athalye, A. M. et al., J. Chromatography, 589 (1992)

71-85).

DEPR:

The terminology "composite media" is intended to cover all combinations of

physical (or support) structures or compounds, including mineral oxide

matrices, mineral oxide matrices whose interior and exterior surfaces are

substantially covered by a thin protective polymer surface coating, metal

matrices, and polymeric matrices such as polystyrenes, with chromatographic

resins such as those made from chemical substances known to be useful for the

preparation of chromatographic separation adsorbents, including polymerized

vinyl monomers that contain chromatographically active substituents. The

constituents of the composite media can include structures and chromatographic

resins made separately or formed together, such as in block copolymers.

DEPR:

An important figure of merit that facilitates understanding the present

invention is the formula for flux enhancement factor ##EQU4##

wherein $D_{sub.s}$ is the experimentally determined effective intraparticle diffusivity of the biological molecule to be separated, τ is the tortuosity factor of the porous chromatography support, $D_{sub.f}$ is the diffusivity in free solution of the biological molecule, and $\epsilon_{sub.p}$ is the void volume of the composite support matrix measured in the absence of a polymeric gel filling the pores. $D_{sub.s}$ is defined in such a way that the driving force for diffusion in the particle is the gradient in the total protein concentration at that point in the particle, not just the gradient in "free" or "unbound" protein concentration. Thus the flux enhancement factor E is determined by both the characteristics of the chromatography medium e.g. (τ and $\epsilon_{sub.p}$) and the characteristics of the biological molecule to be separated (e.g. $D_{sub.s}$ and $D_{sub.f}$). The variable $D_{sub.s}$ depends upon the biological molecule as well as on the composite media. This flux enhancement factor E represents the ratio of (i) the intraparticle diffusional solute flux to (ii) the intraparticle flux of solute that would be expected to occur if the pores of the composite media support were devoid of the polymeric network.

DEPR:

The second flux enhancement factor E^* takes account of the steric effects of the gel residing within the pores of the composite media. This flux enhancement factor E^* is the ratio of (i) the intraparticle diffusional solute flux to (ii) the intraparticle flux of solute that would occur if the pores of the composite media support were filled with a polymeric network identical to that present in the composite media of the invention save for its being incapable of interacting electrostatically or chemically with the solute of interest. The steric effects of the gel decrease the effective

area open for diffusion in the composite media. A decrease in effective area for diffusion would, in a conventional media, significantly inhibit diffusion in the gel. In the present invention, however, this steric effect of the gel is overcome by the favorable partitioning of solute into the gel; that is, the high intraparticle solute concentration gives rise to an increase in the total driving force for diffusion in the composite media and thus an increase in the total flux into the particle. It may be noted that E and E^* account for the fact that the biological macromolecule must diffuse by a geometrically tortuous route in order to penetrate the particle. Thus, in comparing the enhancement of intraparticle flux with that in free solution, E and E^* account for the longer diffusional distance with the tortuosity parameter τ . Values for τ generally vary between 2 and 6. A value for tortuosity of 2 has been adopted for calculations of E and E^* presented below, because such a value is typical of many chromatographic media, and because adoption of a tortuosity value of 2 results in calculated E and E^* values that are conservative.

DEPR:

The flux enhancement factors E and E^* defined above are designed so as to capture the unexpectedly high mass transfer performance (e.g., intraparticle solute fluxes) of the composite media of the present invention. In particular, the first flux enhancement factor E is meant to compare the observed rate of intraparticle solute mass transfer with the rate of mass transfer that would be predicted were the solute to be diffusing into the "empty" (e.g., gel-free) porous support particle from which the composite media of the present invention is fashioned. One of ordinary skill might reasonably expect the solute to diffuse into the gel-containing composite particle no more

rapidly than it
would diffuse into the "empty" support particle; this expected
rate of mass
transfer would be proportional to the solution-phase solute
concentration
driving force $C_{s,0}$ and to an effective intraparticle solute
diffusivity for
such a gel-free particle given by the following expression:
##EQU5## Inasmuch
as E is the ratio of a term proportional to the actual
intraparticle flux
(i.e., $D_{s,s} \cdot q_{s,0}$) to a comparable term proportional
to the flux
that would be predicted for a gel-free porous support particle,
then --absent
other considerations (i.e., steric exclusion by the gel)--the
expected value of
 E would be of order unity or less. In contrast, the flux
enhancement factor E
achievable through the present invention can be substantially
greater than
unity--and significantly greater than the E values provided by
prior-art
media--as shown in more detail below.

DEPR:

The degree of flux enhancement achievable through the present
invention is
particularly unexpected when the steric exclusion of the polymer
network or gel
present within the pores of the composite media support is taken
into account
in the prediction of the expected intraparticle solute flux. In
particular,
the presence of the gel within the support particle's pores
reduces the porous
volume (or, alternatively, the effective area for diffusion) that
is accessible
to molecules diffusing within it. It is this steric effect of
the gel in
reducing this expected intraparticle flux which the second flux
enhancement
factor E^* is designed to capture. In particular, one of ordinary
skill would
expect the solute to diffuse into the composite particle no more
rapidly than
it would diffuse into a support particle filled with a
non-interactive gel; in
this case, the expected rate of mass transfer would again be
proportional to

the solution-phase solute concentration driving force $C_{sub.0}$
--but the
effective intraparticle diffusivity would be smaller, as given by
the following
expression: $\epsilon_{p} \cdot D_{sub.s}$ where ϵ_{p} is the
species-dependent inclusion
porosity. Inasmuch as E^* is the ratio of a term proportional to
the actual
intraparticle flux (i.e., $D_{sub.s} \cdot q_{sub.0}$) to a term
proportional to the
predicted or expected intraparticle flux taking steric exclusion
of solute by
the gel into account, one of ordinary skill might reasonably
anticipate that
composite media would provide E^* values of order unity or lower.
However, the
composite media of the present invention are characterized by
much larger E^*
values that differentiate them from prior-art composite media.

DEPR:

The parameter τ is the tortuosity factor characteristic of
the skeletal
matrix of the composite media support particle. The parameter
 τ is
related to the additional distance that a solute has to diffuse
in the tortuous
pores of the support as compared to how far it would have to
diffuse in
"straight" pores or in free solution. Because the pore walls of
the composite
media of the support particle do not allow diffusion through
them, the solute
cannot diffuse directly from some point A to some point B;
instead the solute
has to move from point A somewhat away from a straight line to
point B to point
C--and only then to point B. In contrast, in free solution, the
solute can
diffuse directly from point A to point B. This tortuosity
therefore decreases
the effective concentration gradient and thus the speed at which
the biological
molecule to be separated diffuses into the particle skeleton,
thus reducing the
performance of the media. Values for the tortuosity parameter
 τ can be
determined by first measuring solute mass transfer rates of a
very small solute
in a porous support particle and then extracting an effective

intraparticle diffusivity from these rates, taking the support geometry and the solution-phase solute concentration driving force into proper account. Next, one measures the void fraction ϵ_p of the porous support (e.g., by mercury porosimetry or other standard method as discussed further below). The tortuosity parameter τ is then obtained by dividing the product of the solution-phase solute diffusivity D_f (see below) times the support particle porosity ϵ_p by the effective intraparticle solute diffusivity. This tortuosity factor can be measured with a small diffusive probe, or by comparing the intraparticle diffusivity of a series of variously sized molecules. See Coffman, J. L., Ph.D. Thesis, University of Wisconsin, 1994. τ usually has a value of from about 2 to about 6 for many chromatographic materials (See Coffman, J. L., Ph.D. Thesis, University of Wisconsin, 1994). A conservative τ value of 2 has been assumed in E and E* calculations presented here.

DEPR:

ϵ_p is the fractional void volume of the skeleton of the composite media, which also represents the effective area open for diffusion of the biological molecule of interest in the skeleton of the composite.

It represents the fraction of the volume of the support particle occupied by pores before those pores are at least partially filled with the polymer network. This parameter ϵ_p can be measured by mercury intrusion by those skilled in the art.

DEPR:

The parameter ϵ_p^* is the species-dependent inclusion porosity or species-dependent void volume fraction of the composite media with the polymer network present within the pores. It measures the volume fraction of the

composite particle (i.e., gel plus support particle) that is accessible to the solute and is related to the partition coefficient of the biological molecule under nonbinding conditions, that is, conditions under which the solute interacts with the polymer network or gel within the pores of the support exclusively via steric interactions. This factor, ϵ_p , also represents the effective area open for diffusion of the biological molecule of interest in the supported gel.

DEPR:

For composite media, ϵ_p derives from two effects. One is the fact that the support or skeleton of the composite media takes up space in the media, leaving only the void volume ϵ_p accessible to solute. Secondly, this porous volume contains gel in the composite media of the invention, and the polymer molecules of this gel can be arranged in such a way as to create a fine three-dimensional mesh or network. In the media of the present invention, this effective mesh size is very small, on the order of the size of biological molecules of interest. Since the mesh size is so small, many biological molecules of interest do not fit well into, and are thus at least partially excluded from, the effective pores or spaces between the polymer molecules comprising the mesh, which further and significantly reduces the solute accessible volume and thus makes ϵ_p significantly smaller than the support void fraction ϵ_p . This steric exclusion by the polymeric network can be theoretically described by the so-called Ogston equation $\epsilon_p = \left(1 - \frac{a}{a_s}\right)^2$ where a is the Stokes radius of the biological molecule of interest, a_s is the effective radius of a polymer strand, and ϕ is the volume fraction of the polymer forming the network. For the present invention, ϕ is the order of several percent (e.g.

preferably, 0.03 to 0.20); $a_{sub.f}$ is equal to 6.5 \AA . for polyacrylamide gels. The Stokes radius for globular proteins can be obtained from the correlation

DEPR:

The effective intraparticle diffusivity can be measured in several ways, including a batch uptake method, a shallow bed chromatography method, and by analyzing breakthrough curves on a long column as discussed further below. The parameter $q_{sub.0}$ is the intraparticle concentration of the biological macromolecule of interest in the composite media at equilibrium with a solution-phase solute concentration of $C_{sub.o}$.

DEPR:

Table I compares values of E determined for some embodiments of the present invention (first three entries) with values of E calculated for certain prior art composite media systems. Methods for the determination or estimation of the various parameters that comprise E are discussed in more detail in the Examples section below.

DEPR:

The unexpectedly superior mass transfer performance of the present invention is better described by taking into account the expected steric effects of the gel present within the pores of the composite medium. This is done through the diffusional flux enhancement factor E^* where the effect of steric exclusion of the solute by the gel on the expected intraparticle solute flux is included in the species-dependent inclusion porosity $\epsilon_{sub.p}$ as described above. E^* quantitates the degree of flux enhancement through the area that is actually open for diffusion through the supported gel, as opposed to E , where the open area for diffusion is presumed to be the entire open area of the skeleton or porous support. In accordance with the present invention,

E* is generally above about 3 and is preferably at least about 50 and most preferably at least about 300.

DEPR:

Table II compares values of E* determined for some embodiments of the present invention (first three entries) with values of E* calculated for certain prior art composite media systems.

DEPR:

The composite media of the present invention are superior to prior-art composite media by virtue of their enhanced intraparticle mass transfer rates. That is, the values of E and E* determined for the HyperD media of the present invention are typically an order of magnitude or more higher than those of prior-art supported-gel composite media (see Tables I and II).

DEPR:

The composite media of the present invention are also superior to prior-art non-composite media (e.g., unsupported gels, porous silica, etc.) not only by virtue of their high mass transfer efficiency but also by virtue of their improved rigidity and other mechanical properties which enable their use in high-speed chromatographic operations. Table III.A shows the values of the flux enhancement factor E computed for non-composite media, while Table III.B shows the calculated values of the second flux enhancement factor E* for the same prior art media.

DEPR:

Without wishing to be limited by theory, it is currently believed that the media of the present invention provide large values for E and E* compared to prior conventional media because of enhanced mobility of the biological molecules of interest inside the media. This enhanced mobility results, it is believed, from the fact that when the biological molecule of interest interacts

with and/or adsorbs or binds to the media of the present invention, the biological molecule remains appreciably mobile. Moreover, because the binding capacity $q_{sub.0}$ is high, the concentration of bound but mobile protein (or other biological on non-biological solute) can be high--leading to large and steep intraparticle concentration gradients that give rise to large intraparticle diffusional fluxes. In many conventional media, when the biological molecule of interest adsorbs or binds to the media, the biological molecule for the most part ceases to move and remains essentially stationary. This decreases the total flux of biological molecules into conventional sorbents as compared to the flux of molecules into the composite media described herein.

DEPR:

Under strong adsorption conditions, it may be that only one molecule out of one or several thousands of molecules will be unbound or unadsorbed in both conventional media and in the composite media described herein. This means that under strong binding conditions favorable for separating biological molecules, perhaps only about one of a thousand solute molecules are appreciably mobile in conventional media, the rest being bound or adsorbed to the matrix in a relatively immobile or stationary condition. Under similar binding conditions, however, a majority if not nearly all of the solute molecules within the media of the present invention are mobile, whether or not they are interacting with the polymeric network. The consequence of this is that in the media of the present invention, the driving force for intraparticle diffusion is much larger, as large as a thousand or more times larger, than is the driving force for intraparticle diffusion in conventional media. In fact, the driving force for diffusion in the media of the present

invention can approach the gradient in the total intraparticle solute concentration. The driving force for most conventional media, on the other hand, is limited to the unbound solute concentration, which is significantly smaller. Since the driving force for diffusion in the media of the present invention is so much higher, the flux is significantly greater into the present media than into conventional media. That the (biological) solute molecule of interest can remain mobile while interacting with and/or adsorbed or bound to the chromatographic media of the present invention to the extent described herein was entirely unexpected.

DEPR:

The total protein concentration in the supported-gel media, the gradient of which is the driving force for mass transfer in the present invention, is a function of the static capacity of the media for the particular solute of interest. This static capacity depends on, among other factors, the salt concentration, the pH, the properties of the gel, and the solute species. When the static sorption capacity is significantly higher than the free solution concentration, then the rate of intraparticle mass transfer in the above-mentioned composite particle will be largely independent of the external solution concentration. One consequence of this is that the flux enhancement factors E and E^* characteristic of the invention get progressively higher as the external solution concentration decreases. This is due to the fact that the flux enhancement factors E and E^* compare the rate of intraparticle diffusion within the gel-containing composite particle of the present invention with the rates of diffusional mass transfer within, respectively, (i) the porous support particle devoid of gel or (ii) the porous support particle

containing gel that interacts only sterically with the solute of interest. Since the driving force decreases with decreasing external solution concentration in the latter instances, whereas in the present media the driving force remains relatively independent of the external solution concentration, E and E^* increase with decreasing $C_{sub.0}$. This effect makes the composite media of the present invention valuable for efficiently adsorbing biological macromolecules from dilute solutions of the biological macromolecule of interest. Dilute solutions of biological macromolecules dominate the biotechnology industry, as fermentation, for instance, produces relatively low concentrations of the biological macromolecule of interest. Solute concentrations vary widely from application to application, but $C_{sub.0}$ values are generally at least 1 $\mu\text{g/mL}$, more typically are on order 10 $\mu\text{g/mL}$ or greater, and preferably are on order 100 $\mu\text{g/mL}$ and larger. Similar considerations apply to the use of the composite media in recovery of valuable metals from dilute solutions and in the capture of environmental pollutants, and other applications.

DEPR:

Without wishing to be limited by theory, it is believed that the high capacity of the present composite media and the high mass transfer rates in that media are aided by the flexibility of the gel polymer network incorporated into the rigid porous support. The flexibility of the gel allows for the solute to penetrate the gel by either the solute pushing aside the polymeric network gel in order to get by, or by the gel molecules spontaneously moving to form a hole into which the solute can move. This is particularly important where the effective diameter of the solute is large in comparison with the characteristic length between the polymer chains that form the three-dimensional

polymeric gel network--e.g., where the solute is a biological macromolecule like a protein, a polysaccharide, a polynucleotide or others. It is further believed that the three-dimensional nature of the gel and the small mesh size of the gel contribute to the high capacity of the media and to the ability of large solutes (e.g., biological macromolecules) to move even while interacting with the active portions, e.g., ion exchange sites or affinity sites, of the gel. The fineness of the polymeric network mesh also means that these sites are in close proximity, such that molecules can move from site to site quickly, without having to desorb and/or move very far between sites.

DEPR:

Without wishing to be limited by theory, one can speculate that the confinement of the polymeric gel network of the present invention within the pores of the rigid porous support matrix may also be important to the operation of the invention--in particular, to the features of high binding capacity and high intraparticle diffusive mass transfer rates. If the polymeric gel network of the present invention were "free" or unconfined--as opposed to being confined within the porous volume of the support matrix of the present composite media, then the as polymerized gel swells or increases in volume several-fold when exposed to dilute aqueous solutions of the sort normally encountered in biochromatography; this swelling results from, e.g., in ion-exchange gel media or in affinity media where the affinity to the biological macromolecule of interest is to some extent ionic (such as heparin affinity or lysine affinity for example), the repulsion of fixed charges of like sign on the polymeric network. This swelling effectively "dilutes" the binding sites (thus reducing binding capacity), and, under certain circumstances, may make it necessary for

a solute to disassociate from one binding site before diffusing to and interacting with another. In contrast, with the confined gel of the present invention, the polymeric network cannot swell, despite the strong interaction of the fixed ionic charges. As a consequence, binding capacity remains high. Moreover, the regions of ionic interaction overlap, and a solute of opposite charge (such as a biological macromolecule) can move freely within the entire polymer network while interacting electrostatically with more than one ionic group or affinity site of the three dimensional polymeric network. That is, a sorbed solute molecule may not have to dissociate from one binding "site" before diffusing to and interacting with another, since the binding "sites" are not necessarily discrete in the polymeric network contained within the composite media of the present invention. This has clearly unexpected advantages in terms of enhanced intraparticle mass transfer rates.

DEPR:

In a preferred embodiment, the present invention provides a passivated composite sorbent particle comprising a porous solid matrix having interior and exterior surfaces and innate (i.e., inherently present) groups that render the matrix susceptible to undesirable non-specific interaction with biological molecules, and a polymer network derived from a passivation mixture comprising effective amounts of a main monomer, a passivating monomer different from the main monomer, and a crosslinking agent, the mixture having been allowed to come into intimate contact with the surfaces of the matrix for a sufficient period of time such that on polymerization of the mixture the innate groups of the matrix become deactivated, resulting in the minimization or substantial elimination of the above-mentioned undesirable non-specific interactions.

DEPR:

The passivated composite media of the present invention are further characterized by reversible high sorptive capacity and high intraparticle diffusive mass transfer rates for biological molecules including proteins. Furthermore, the passivated composite media of the present invention enjoy exceptional chemical stability on exposure to strongly acidic or alkaline media and/or strong oxidizing solutions such as those that are frequently utilized during cleaning of industrial manufacturing equipment.

DEPR:

While it has been discovered that this process of depositing protective polymer coatings upon the porous surfaces of mineral oxide (and particularly silica) matrices can significantly stabilize these materials by sharply reducing their rates of chemical leaching, the approach has the important disadvantage of rendering the porous surfaces of the coated and protected matrices hydrophobic and thus prone to cause excessive non-specific binding of proteins by adsorption. (This is precisely the same problem noted above in connection with entirely polymeric porous support matrices.) However, this problem can be successfully addressed by the methods of the present invention in the same way as the non-specific binding of strictly polymeric support matrices can be reduced --i.e., by passivation in a process of oriented polymerization. More particularly, these composite chromatographic media (i.e., supports comprised of mineral oxide substrates that have been stabilized by the application of thin protective polymer coatings) can be passivated against excessive non-specific binding by incorporating passivating ("neutralizing") monomers capable of associating with and consequently deactivating innate non-polar hydrophobic groups exposed on the matrix surface. The

passivating monomers
useful in this embodiment of the present invention adsorb upon
(and
consequently cover) the hydrophobic groups on the surface by
virtue of their
containing long-chain saturated hydrocarbons, olefinic
hydrocarbon groups,
aromatic groups, or like hydrophobic domains that interact with
and become
appreciably bound to their hydrophobic counterparts on the matrix
surface as a
consequence of the hydrophobic-hydrophobic interaction existing
between them.

DEPR:

The size exclusion limit of the composite media varies somewhat
but generally
falls in the range of about 500 to about 2,000,000 Daltons,
preferably, 50,000
to about 500,000. The sorptive capacity can also be manipulated,
depending on
the amount of main monomer incorporated in the polymer network,
and ranges
between about 1 milligram to about 300 milligrams of solute or
biological
molecule per unit volume (mL) of media--preferably at least about
50 mg/mL, and
most preferably about 100 mg/mL.

DEPR:

Eluent solutions suitable for use in the present invention are
well known to
those of ordinary skill in the art. For example, a change in
ionic strength,
pH or solvent composition may be effective in "stepwise" elution
processes.
Alternately, eluent solutions may comprise a salt gradient, a pH
gradient or
any particular solvent or solvent mixture that is specifically
useful in
displacing the preselected biological molecule. Such methods are
generally
known to those engaged in the practice of protein chromatography.

Still
another object of the present invention relates to a
chromatographic method for
the separation of biological molecules comprising passing a
sample containing a
mixture of biological molecules through a column packed with the
composite

media disclosed herein.

DEPR:

By virtue of their superior mass transfer characteristics and high binding capacity, it is anticipated that the composite particles of the present invention will also find use outside the field of biochromatography--both as adsorbents (in chromatographic and other types of sorption processes, e.g., for the recovery of environmental pollutants, valuable metals, etc.) and as solid-phase supports for the conduct of chemical reactions, the immobilization of reactants and catalysts, and the capture of reaction products.

DEPR:

In another embodiment, the polymerization step can take place in the presence of a pore inducer. The pore inducers of the present invention allow polymerization of the gel to take place while maximizing the accessibility of the interior volume of the composite media. Suitable pore inducers, also referred to as porogens, used in the present invention include, but are not limited to, polyethylene glycols, polyoxyethylenes, polysaccharides such as dextran, and polar solvents. Polar solvents include those commonly used in chemical synthesis or polymer chemistry and known to those skilled in the art. Suitable polar solvents include alcohols, ketones, tetrahydrofuran, dimethylformamide, and dimethylsulfoxide. Preferred polar solvents are ethanol, methanol, dioxane, and dimethylsulfoxide.

DEPR:

In all cases, i.e., whether the porous matrix is comprised of a mineral oxide, a polymer-coated and thus stabilized mineral oxide, or a polymer, the polymerization process of the present invention creates a three-dimensional lattice or crosslinked polymer network that extends away from the pore-wall surfaces of the porous solid matrix. Again, not wishing to be

limited by theory, it is believed that this polymer network is comprised of a thin passivating region or layer that ideally interacts with the surface of any non-specific adsorption sites of the solid support (e.g., silanols in the case of silica) covalently linked with a three-dimensional structural polymer lattice that can (but need not necessarily) substantially fill the porous volume. In a preferred embodiment, the three-dimensional shape of the polymer lattice is believed to be substantially identical to the shape of the pore which it fills (see FIG. 5), with any passivating layer oriented adjacent to and continuous (i.e., covalently linked) to the three-dimensional polymer lattice that extends away from the matrix surface. This configuration prevents "neutralizing" or "deactivating" pieces of the polymer network from eluting from the composite media during regular use--for example, when it is exposed to vigorous washing or cleaning conditions, such as high acidic pH, high alkaline pH, high ionic strength, and strong oxidizing conditions. This crosslinked polymer network creates a novel chromatographic sorbent which can then be used, for example, in a process for separating and purifying various biomolecules, including macromolecules.

DEPR:

Indeed, it has been surprisingly discovered that the composite media of the present invention manifest chromatographic characteristics that are unparalleled under several criteria, particularly in terms of dynamic sorptive capacity as a function of flow rate and high intraparticle mass transfer rates. In particular, whereas the great majority of porous materials suffer a marked decrease in useful sorptive capacity as flow rates increase (e.g., at flow rates of about 50 cm/hr or greater), the passivated porous supports of the

present invention show little decrease in useful sorptive capacity from a static condition up to flow rates approaching several hundred centimeters per hour. Compare, for example, the behavior of prior art "gel"-type materials with the supports of the present invention, as illustrated in the graphs of FIG. 3A, 3B, and 4 (described further in Example 16).

DEPR:

Moreover, the absolute capacities of the composite media of the present invention are considerably greater than those attained with other types of chromatographic media (e.g., Spherodex.TM.) Thus, as shown in FIG. 4, a plot of the absolute capacity vs. flow rate of various chromatographic media unambiguously shows that the composite media of the present invention combines a very high absolute sorption capacity (expressed as mg/mL) with a relative insensitivity to solution flow rates.

DEPR:

It is believed, without wishing to be limited by theory, that a flexible lattice structure comprised primarily of polymeric chains of repeating main monomer units is formed within the pores of the porous solid matrix. Very significantly, it is believed that the areas of the composite media available for desirable reversible interaction with biological molecules are not confined to the regions immediately adjacent to the surface of the pore as is the case when thin, substantially two-dimensional coatings are applied to porous surfaces in the manner of Steuck (U.S. Pat. No. 4,618,533) and Varady et al. (U.S. Pat. No. 5,030,352) as discussed in the Background of the Invention section above. Rather, it is believed that the gel-type polymeric network of the present invention extends outwardly into the pore volume itself in the manner of a three-dimensional preferably but not necessarily pore-filling

lattice, as opposed to a two-dimensional coating limited strictly to the pore wall surface area. A schematic diagram of such a structure, as it is thought to exist, is illustrated in FIG. 5, where a biological molecule of interest (depicted as a spherical object) is also shown interacting with the lattice. Furthermore, the presence of porogens (pore-inducers) in the passivation mixture is believed to promote creation of this three-dimensional polymer network.

DEPR:

It is further thought that such an extended polymer network contributes not only to the unusually high absolute sorptive capacity of the composite media of the invention as measured under static (i.e., no flow) conditions, but also permits rapid intraparticle mass transfer by diffusion and thereby allows the present invention to maintain high sorptive capacities largely independent of solution flow rates. It is thought that perhaps the flexible nature of the three-dimensional polymer network allows biological molecules to rapidly penetrate the polymer lattice and thereby efficiently interact with sorptive groups in the polymer network of the passivated porous support while maintaining their mobility even at high solution flow rates. The rapid and efficient mass transfer of biomolecules into and through this network avoids the decrease in useful or dynamic sorption capacity and resolution that are typical of conventional chromatographic media. With these conventional media, diffusion in the pores of the particle and/or materials coated thereupon or within them can be slow, leading to poor mass transfer rates and poor efficiency of the chromatographic process.

DEPR:

Thus, a method of performing chromatographic separations characterized by high

sustained sorptive capacity relatively independent of flow rate and rapid, efficient intraparticle mass transfer is achieved with composite media of the present invention, which media include a flexible three-dimensional network or lattice of crosslinked polymer chains extending within and throughout the pores of the support matrix.

DEPR:

The separation and purification process usually involves at least two steps.

The first step is to charge a packed or fluidized bed column containing the preferably passivated composite adsorbent with a solution containing a mixture of biomolecules, at least one of which it is desired to separate and recover in at least partially purified form. The second step is to pass an eluent solution through the column to effect the release of the biomolecules from the column, thereby causing their separation.

DEPR:

It is thus demonstrated that the level of non-specific adsorption for lysozyme

(a strong cationic protein) is high when the MAPTAC is absent.

The

non-specific adsorption for silica with large surface area (X 075, 100 m.²/g)

is higher (55 mg/mL of resin) than the non-specific adsorption for silica X

015 (25 m.²/g; 15 mg/mL of resin). A certain proportionality exists

between the surface area and the original level of non-specific absorptions.

The amount of MAPTAC to decrease the level of non-specific adsorption down to

zero is also proportional to the surface area available: 1.5% of MAPTAC is

necessary with silica X 015 (25 m.²/g) whereas at least 6% is necessary to

passivate silica X 075 (100 m.²/g).

DEPR:

The passivated resin is then recovered by filtration. The oil is eliminated

with an extensive washing with water containing 0.1-0.5% of a

non-ionic
detergent and then stored in a saline buffer at neutral pH. The
product resin
shows very similar ion-exchange characteristics as those
described in Example
2. Additionally, its sensitivity in strong alkaline media is
much improved as
measured by its weight loss after one night of contact with 0.5M
sodium
hydroxide. The passivated resin of this example lost only about
half as much
weight as an anionic resin prepared from silica having an
unprotected surface
area.

DEPR:

Various polyacrylamide/~~silica~~ composites prepared as described
hereinabove (and
according to U.S. Pat. No. 5,268,097) are used as supports in
accordance with
the present invention in the Examples which follows. These
materials are
referred to hereinafter as S-HyperD F, S-HyperD M, Q-HyperD F,
and Q-HyperD M.
The S-HyperD media is a series of cation exchange chromatography
media of
various particle sizes. The Q-HyperD series is a series of anion
exchange
chromatography media of various particle sizes. The "F" and "M"
suffixes refer
to HyperD media particle sizes, with F indicating nominal media
particle
diameters of 35 micrometers and M indicating nominal media
particle diameters
of 60 micrometers.

DEPR:

Two different polyacrylamide/silica composites were prepared as
described above
and are referred to hereinafter as Q-HyperD F and Q-HyperD M. The
water
contents of the Q-HyperD F and M media samples were determined.
The particle
density of hydrated Q-HyperD media was measured to be 1.424 g/cm.
The particle
sizes and particle size distributions were measured optically.

DEPR:

A polyacrylamide/silica composite was prepared as described above
and is

referred to hereinafter as Q-HyperD M. The water content of the Q-HyperD M media samples was determined. The particle density of hydrated Q-HyperD M media was calculated to be 1.424 g/cm.³. The particle size and particle size distribution were measured optically.

DEPR:

A polyacrylamide/silica composite was prepared as described above and is referred to hereinafter as Q-HyperD M. The water content of the Q-HyperD M media samples was determined. The particle density of hydrated Q-HyperD M media was calculated to be 1.424 g/cm.³. The particle size and particle size distribution were measured optically.

DEPR:

Polyacrylamide/silica composites were prepared as described above and are referred to hereinafter as Q-HyperD F and as Q-HyperD M.

DEPR:

Polyacrylamide/silica composites were prepared as described above and are referred to hereinafter as Q-HyperD F and as Q-HyperD M.

DEPR:

Polyacrylamide/silica composites were prepared as described above and are referred to hereinafter as Q-HyperD F and as Q-HyperD M.

DEPR:

Substitution of these and other parameters in the above expression yields bed void volumes ϵ of 0.5, 0.48, 0.49, and 0.5 for four columns packed with the HyperD composite media of the present invention.

DEPR:

A polyacrylamide/silica composite was prepared as described above and is referred to hereinafter as Q-HyperD F.

DEPR:

The species-dependent void volume of the media in the present invention can be adequately predicted by applying the Ogston equation to describe

exclusion by
the polymer network of the composite media--modifying the Ogston
equation to
take into account the volume taken up by the skeleton or porous
support
particle that forms the base matrix for the composite media of
this invention.
The Ogston equation so modified is shown below: ##EQU16## where a
is the Stokes
radius of the biological molecule of interest, $a_{\text{sub.f}}$ is the
effective radius
of a strand of polymer forming the gel network, and ϕ is the
volume
fraction of the polymer forming the network. For the HyperD
embodiment of
present invention based on a silica-supported polyacrylamide gel,
the polymer
volume fraction ϕ is equal to 0.105, and the effective
radius of a strand
of polyacrylamide is 6.5 \AA . The Stokes radius for globular
proteins can be
obtained from the correlation

DEPL:

The composite media

DEPL:

Preparation of an Anion-Exchange Resin Using a Surface-Protected
(i.e.,
Pre-coated) Silica Passivated Porous Support

DEPL:

Determination of Ion-Exchange and Protein Sorption Capacity of
Preparation of
Anion-Exchange Resins Based on Passivated Porous Silica Support
of Different
Surface Areas

DEPL:

Determination of adsorption capacity (BSA on
polyacrylamide/silica composites)

DEPL:

Determination of adsorption capacity (ovalbumin on
polyacrylamide/silica
composite)

DEPL:

Determination of adsorption capacity (α -lactalbumin on
polyacrylamide/silica composite)

DEPL:

From the agreement between ϵ_{sp} values for Dextran T-40 and BSA, it can be seen that ϵ_{sp} is independent of salt concentration between salt concentrations up to at least 1000 millimolar salt. It can also be seen from ϵ_{sp} values for blue dextran (a very large solute, having a molecular weight of approximately 2,000,000) and BSA that molecules as large or larger than BSA have ϵ_{sp} values less than about 0.01. The fact that these ϵ_{sp} values are generally much smaller than unity illustrates that, under nonbinding/noninteractive conditions, the degree of steric exclusion of solute by the supported polymeric gel network of the present invention is indeed appreciable. Given this steric effect, it is thus particularly unexpected that the rates of intraparticle diffusive mass transfer can be as large as observed--as exemplified by the large values of the flux enhancement factor E^* calculated for composite media of the present invention.

CLPR:

2. The method of claim 1, wherein said composite media provides a larger dynamic capacity for said macromolecule at initial feed concentrations of less than about 2 milligrams per milliliter than the dynamic capacity provided by the same media for the same macromolecule at initial feed concentrations of greater than about 2 milligrams per milliliter.

CLPR:

6. The method of claim 1, which further comprises selecting the composite media to be a chromatographic media so that said biological macromolecules can be separated by chromatography.

CLPV:

selecting a composite media comprising a porous support comprising voids

containing a polymeric network, wherein the composite media has a size exclusion limit of about 500 Daltons to about 2,000,000 Daltons and provides a value of a flux enhancement factor E greater than about 3, as determined by the equation $E = \frac{D_s}{D_p}$ in which D_s is the experimentally measured effective intraparticle diffusivity of a biological molecule of interest,

CLPV:

ϵ_p is the fractional void volume of the porous support of the composite media,

CLPV:

τ is the tortuosity of the porous support of the composite media, and

CLPW:

passing, at a flow rate of at least about 50 cm/hr, a sample containing a mixture of biological macromolecules including a biological macromolecule of interest, wherein initial C_0 is less than about 2 milligrams per milliliter, through a column packed with the composite media which provides a larger dynamic capacity for said biological macromolecule of interest at a low concentration than the dynamic capacity provided by the same media for the same macromolecule at a higher concentration; and

CCXR:

210/198.2

DOCUMENT-IDENTIFIER: US 5738783 A

TITLE: Liquid chromatograph having a micro- and semi-micro column

DEPR:

In the present invention, porous media commonly used in liquid chromatography

such as silica gel, alumina, porous glass beads, zeolite, hydroxyapatite and

graphite may be used as the packing material of the column 40.

Alternatively,

it is possible to use composite powders formed of a resin core covered by

inorganic powders such as silica gel, titanium dioxide, hydroxyapatite, and the

like. One may use materials such as polyamides, acrylic resins, polyvinyl

alcohols, and the like, for the resin core.

CCOR:

210/198.2

DOCUMENT-IDENTIFIER: US 5672276 A

TITLE: Passivated porous polymer supports and methods for the preparation and use of same

BSPR:

Several previous investigators have sought to passivate various microporous media including membranes and particulate chromatographic supports by applying thin surface coatings to inorganic or organic/polymeric substrates. For example, Steuck, in U.S. Pat. No. 4,618,533, discloses a porous polymeric membrane substrate fashioned from a thermoplastic organic polymer upon which a permanent coating is grafted and/or deposited on the entire membrane surface. The polymerization and crosslinking of the polymerizable monomer upon and within the porous membrane substrate is performed in such a way that a thin coating is deposited upon the entire surface of the porous membrane, including the inner pore walls. Significantly, the porous configurations of the coated, composite membrane structures claimed by Steuck are essentially identical to those of the corresponding uncoated porous membrane substrates, implying that the polymer of Steuck is applied as a thin surface layer or coating that does not interfere with the porosity or flow properties of his composite membranes. Moreover, Steuck does not disclose the concept of a "passivating layer" or the use of monomers capable of functioning as "passivating" monomers within the meaning of the present invention as discussed in more detail below.

BSPR:

While it has been discovered that this process of depositing protective polymer coatings upon the porous surfaces of mineral oxide (and particularly silica) matrices can significantly stabilize these materials by sharply reducing their

rates of chemical leaching, the approach has the important disadvantage of rendering the porous surfaces of the coated and protected matrices hydrophobic and thus prone to cause excessive non-specific binding of proteins by adsorption. (This is precisely the same problem noted above in connection with entirely polymeric porous support matrices.) However, this problem can be successfully addressed by the methods of the present invention in the same way as the non-specific binding of strictly polymeric support matrices can be reduced--i.e., by passivation in a process of oriented polymerization. More particularly, these composite chromatographic supports (i.e., supports comprised of mineral oxide substrates that have been stabilized by the application of thin protective polymer coatings) can be passivated against excessive non-specific binding by incorporating passivating ("neutralizing") monomers capable of associating with and consequently deactivating innate non-polar hydrophobic groups exposed on the matrix surface. The passivating monomers useful in this embodiment of the present invention adsorb upon (and consequently cover) the hydrophobic groups on the surface by virtue of their containing long-chain saturated hydrocarbons, olefinic hydrocarbon groups, aromatic groups, or like hydrophobic domains that interact with and become appreciably bound to their hydrophobic counterparts on the matrix surface as a consequence of the hydrophobic-hydrophobic interaction existing between them. Typically, the present invention utilizes base matrices having the following characteristics: an initial average particle size ranging from about 5 to about 1000 microns; an initial porous volume ranging from about 0.2 to about 2 cm.³/gram; an initial surface area ranging from about 1 to about 800 m.²/gram; and an initial pore size ranging from about 50 to about 6000

angstroms. Preferably, the base matrix is characterized by: an initial average particle size ranging from about 10 to about 300 microns, although passivated supports having narrow particle size ranges, such as about 15-20, about 15-25, about 30-45, about 50-60, about 80-100, and about 100-300 microns, are most preferred. Preferred ranges for other characteristics include an initial porous volume ranging from about 0.8 to about 1.2 cm.³ /gram; an initial surface area ranging from about 10 to about 400 m.² /gram; and an initial pore size ranging from about 1000 to about 3000 angstroms. The density of the porous solid matrix obviously varies with its chemical nature, being higher for mineral oxide (e.g., silica) substrates and lower for polymeric ones (e.g., polystyrene).

DEPR:

It is thus demonstrated that the level of non-specific adsorption for lysozyme

(a strong cationic protein) is high when the MAPTAC is absent. The

non-specific adsorption for silica with large surface areas (X 075, 100 m.² /g)

is higher (55 mg/ml of resin) than the non-specific adsorption for silica X

015 (25 m.² /g; 15 mg/ml of resin). A certain proportionality exists

between the surface area and the original level of non-specific absorptions.

The amount of MAPTAC to decrease the level of non-specific adsorption down to

zero is also proportional to the surface area available: 1.5% of MAPTAC is

necessary with silica X 015 (25 m.² /g) whereas at least 6% is necessary to

passivate silica X 075 (100 m.² /g).

DEPR:

The passivated resin is then recovered by filtration. The oil is eliminated

with an extensive washing with water containing 0.1-0.5% of a non-ionic

detergent and then stored in a saline buffer at neutral pH. The product resin

shows very similar ion-exchange characteristics as those described in Example 2. Additionally, its sensitivity in strong alkaline media is much improved as measured by its weight loss after one night of contact with 0.5M sodium hydroxide. The passivated resin of this example lost only about half as much weight as an artionic resin prepared from silica having an unprotected surface area.

DEPL:

Preparation of an Anion-Exchange Resin Using a Surface-Protected (i.e., Pre-coated) Silica Passivated Porous Support

DEPL:

Determination of Ion-Exchange and Protein Sorption Capacity of Preparation of Anion-Exchange Resins Based on Passivated Porous Silica Support of Different Surface Areas

CCXR:

210/198.2

DOCUMENT-IDENTIFIER: US 5599453 A

TITLE: Passivated porous supports and methods for the preparation and use of same

BSPR:

Several previous investigators have sought to passivate various microporous media including membranes and particulate chromatographic supports by applying thin surface coatings to inorganic or organic/polymeric substrates. For example, Steuck, in U.S. Pat. No. 4,618,533, discloses a porous polymeric membrane substrate fashioned from a thermoplastic organic polymer upon which a permanent coating is grafted and/or deposited on the entire membrane surface. The polymerization and crosslinking of the polymerizable monomer upon and within the porous membrane substrate is performed in such a way that a thin coating is deposited upon the entire surface of the porous membrane, including the inner pore walls. Significantly, the porous configurations of the coated, composite membrane structures claimed by Steuck are essentially identical to those of the corresponding uncoated porous membrane substrates, implying that the polymer of Steuck is applied as a thin surface layer or coating that does not interfere with the porosity or flow properties of his composite membranes. Moreover, Steuck does not disclose the concept of a "passivating layer" or the use of monomers capable of functioning as "passivating" monomers within the meaning of the present invention as discussed in more detail below.

BSPR:

While it has been discovered that this process of depositing protective polymer coatings upon the porous surfaces of mineral oxide (and particularly silica) matrices can significantly stabilize these materials by sharply reducing their

rates of chemical leaching, the approach has the important disadvantage of rendering the porous surfaces of the coated and protected matrices hydrophobic and thus prone to cause excessive non-specific binding of proteins by adsorption. (This is precisely the same problem noted above in connection with entirely polymeric porous support matrices.) However, this problem can be successfully addressed by the methods of the present invention in the same way as the non-specific binding of strictly polymeric support matrices can be reduced--i.e., by passivation in a process of oriented polymerization. More particularly, these composite chromatographic supports (i.e., supports comprised of mineral oxide substrates that have been stabilized by the application of thin protective polymer coatings) can be passivated against excessive non-specific binding by incorporating passivating ("neutralizing") monomers capable of associating with and consequently deactivating innate non-polar hydrophobic groups exposed on the matrix surface. The passivating monomers useful in this embodiment of the present invention adsorb upon (and consequently cover) the hydrophobic groups on the surface by virtue of their containing long-chain saturated hydrocarbons, olefinic hydrocarbon groups, aromatic groups, or like hydrophobic domains that interact with and become appreciably bound to their hydrophobic counterparts on the matrix surface as a consequence of the hydrophobic-hydrophobic interaction existing between them. Typically, the present invention utilizes base matrices having the following characteristics: an initial average particle size ranging from about 5 to about 1000 microns; an initial porous volume ranging from about 0.2 to about 2 cm.³/gram; an initial surface area ranging from about 1 to about 800 m.²/gram; and an initial pore size ranging from about 50 to about 6000

angstroms. Preferably, the base matrix is characterized by: an initial average particle size ranging from about 10 to about 300 microns, although passivated supports having narrow particle size ranges, such as about 15-20, about 15-25, about 30-45, about 50-6, about 80-100, and about 100-300microns, are most preferred. Preferred ranges for other characteristics include an initial porous volume ranging from about 0.8 to about 1.2 cm.sup.3 /gram; an initial surface area ranging from about 10 to about 400 m.sup.2 /gram; and an initial pore size ranging from about 1000 to about 3000 angstroms. The density of the porous solid matrix obviously varies with its chemical nature, being higher for mineral oxide (e.g., silica) substrates and lower for polymeric ones (e.g., polystyrene).

DEPR:

It is thus demonstrated that the level of non-specific adsorption for lysozyme

(a strong cationic protein) is high when the MAPTAC is absent.

The

non-specific adsorption for silica with large surface areas (X 075, 100 m.sup.2

/g) is higher (55 mg/ml of resin) than the non-specific adsorption for silica X

015 (25 m.sup.2 /g; 15 mg/ml of resin). A certain proportionality exists

between the surface area and the original level of non-specific absorptions.

The amount of MAPTAC to decrease the level of non-specific adsorption down to

zero is also proportional to the surface area available: 1.5% of MAPTAC is

necessary with silica X 015 (25 m.sup.2 /g) whereas at least 6% is necessary to

passivate silica X 075 (100 m.sup.2 /g).

DEPR:

The passivated resin is then recovered by filtration. The oil is eliminated

with an extensive washing with water containing 0.1-0.5% of a non-ionic

detergent and then stored in a saline buffer at neutral pH. The product resin

shows very similar ion-exchange characteristics as those described in Example 2. Additionally, its sensitivity in strong alkaline media is much improved as measured by its weight loss after one night of contact with 0.5M sodium hydroxide. The passivated resin of this example lost only about half as much weight as an anionic resin prepared from silica having an unprotected surface area.

DEPL:

Preparation of an Anion-Exchange Resin Using a Surface-Protected (i.e., Pre-coated) Silica Passivated Porous Support

DEPL:

Determination of Ion-Exchange and Protein Sorption Capacity of Preparation of Anion-Exchange Resins Based on Passivated Porous Silica Support of Different Surface Areas

CCXR:

210/198.2

DOCUMENT-IDENTIFIER: US 5593576 A

TITLE: Passivated porous polymer supports and methods for the preparation and use of same

BSPR:

Several previous investigators have sought to passivate various microporous media including membranes and particulate chromatographic supports by applying thin surface coatings to inorganic or organic/polymeric substrates. For example, Steuck, in U.S. Pat. No. 4,618,533, discloses a porous polymeric membrane substrate fashioned from a thermoplastic organic polymer upon which a permanent coating is grafted and/or deposited on the entire membrane surface. The polymerization and crosslinking of the polymerizable monomer upon and within the porous membrane substrate is performed in such a way that a thin coating is deposited upon the entire surface of the porous membrane, including the inner pore walls. Significantly, the porous configurations of the coated, composite membrane structures claimed by Steuck are essentially identical to those of the corresponding uncoated porous membrane substrates, implying that the polymer of Steuck is applied as a thin surface layer or coating that does not interfere with the porosity or flow properties of his composite membranes. Moreover, Steuck does not disclose the concept of a "passivating layer" or the use of monomers capable of functioning as "passivating" monomers within the meaning of the present invention as discussed in more detail below.

BSPR:

While it has been discovered that this process of depositing protective polymer coatings upon the porous surfaces of mineral oxide (and particularly silica) matrices can significantly stabilize these materials by sharply reducing their

rates of chemical leaching, the approach has the important disadvantage of rendering the porous surfaces of the coated and protected matrices hydrophobic and thus prone to cause excessive non-specific binding of proteins by adsorption. (This is precisely the same problem noted above in connection with entirely polymeric porous support matrices.) However, this problem can be successfully addressed by the methods of the present invention in the same way as the non-specific binding of strictly polymeric support matrices can be reduced--i.e., by passivation in a process of oriented polymerization. More particularly, these composite chromatographic supports (i.e., supports comprised of mineral oxide substrates that have been stabilized by the application of thin protective polymer coatings) can be passivated against excessive non-specific binding by incorporating passivating ("neutralizing") monomers capable of associating with and consequently deactivating innate nonpolar hydrophobic groups exposed on the matrix surface. The passivating monomers useful in this embodiment of the present invention adsorb upon (and consequently cover) the hydrophobic groups on the surface by virtue of their containing long-chain saturated hydrocarbons, olefinic hydrocarbon groups, aromatic groups, or like hydrophobic domains that interact with and become appreciably bound to their hydrophobic counterparts on the matrix surface as a consequence of the hydrophobic-hydrophobic interaction existing between them. Typically, the present invention utilizes base matrices having the following characteristics: an initial average particle size ranging from about 5 to about 1000 microns; an initial porous volume ranging from about 0.2 to about 2 cm.³/gram; an initial surface area ranging from about 1 to about 800 m.²/gram; and an initial pore size ranging from about 50 to about 6000

angstroms. Preferably, the base matrix is characterized by: an initial average particle size ranging from about 10 to about 300 microns, although passivated supports having narrow particle size ranges, such as about 15-20, about 15-25, about 30-45, about 50-60, about 80-100, and about 100-300 microns, are most preferred. Preferred ranges for other characteristics include an initial porous volume ranging from about 0.8 to about 1.2 cm.³ /gram; an initial surface area ranging from about 10 to about 400 m.² /gram; and an initial pore size ranging from about 1000 to about 3000 angstroms. The density of the porous solid matrix obviously varies with its chemical nature, being higher for mineral oxide (e.g., silica) substrates and lower for polymeric ones (e.g., polystyrene).

DEPR:

It is thus demonstrated that the level of non-specific adsorption for lysozyme

(a strong cationic protein) is high when the MAPTAC is absent. The

non-specific adsorption for silica with large surface areas (X 075, 100 m.² /g)

is higher (55 mg/ml of resin) than the non-specific adsorption for silica X

015 (25 m.² /g; 15 mg/ml of resin). A certain proportionality exists

between the surface area and the original level of non-specific absorptions.

The amount of MAPTAC to decrease the level of non-specific adsorption down to

zero is also proportional to the surface area available: 1.5% of MAPTAC is

necessary with silica X 015 (25 m.² /g) whereas at least 6% is necessary to

passivate silica X 075 (100 m.² /g).

DEPR:

The passivated resin is then recovered by filtration. The oil is eliminated

with an extensive washing with water containing 0.1-0.5% of a non-ionic

detergent and then stored in a saline buffer at neutral pH. The product resin

shows very similar ion-exchange characteristics as those described in Example 2. Additionally, its sensitivity in strong alkaline media is much improved as measured by its weight loss after one night of contact with 0.5M sodium hydroxide. The passivated resin of this example lost only about half as much weight as an artionic resin prepared from silica having an unprotected surface area.

DEPC:

Preparation of an Anion-Exchange Resin Using a Surface-Protected (i.e., Pre-coated) Silica Passivated Porous Support

DEPC:

Determination of Ion-Exchange and Protein Sorption Capacity of Preparation of Anion-Exchange Resins Based on Passivated Porous Silica Support of Different Surface Areas

CCOR:

210/198.2

DOCUMENT-IDENTIFIER: US 5545317 A

TITLE: Liquid column packing materials and method for making the same

BSPR:

Liquid column (LC) packing materials are usually porous materials which possess adsorptive or catalytic sites on the pore walls. They may be used as packings in columns or as loose material in vessels. LC packing materials typically are porous particles. However, they may instead be fibers or membranes. Porous membranes also provide filtration. When the pore size of the adsorptive or catalytic membrane excludes large proteins, then ultrafiltration is combined with adsorption or catalysis. Membranes may have small pores throughout their mass which exclude protein. Alternatively, the membrane may be a composite of sintered or adhered porous particles, in which case the pores between particles are large whereas the pores within the sintered or adhered particles are small. For example, Kontes Glass Co. markets a thick porous membrane of polyvinyl chloride upon whose large pore walls are attached small porous silica particles. The pores of the silica particles are much smaller than the pores upon whose walls the silica particles are attached.

BSPR:

A second approach to imparting a crosslinked protein coating onto packing materials employs simultaneous contact of glutaraldehyde with a concentrated solution of protein in an unbonded silica slurry in water. Such coated supports have high immobilized protein content and are useful for chromatography of dissolved protein. The object of this approach is to maximize the amount of immobilized protein short of creating an impermeable composite through which liquid could not readily flow. In this approach, the weak adsorption properties of the immobilized protein in the

packing material are useful. See, e.g., M. Tsuboi et al, "Chromatography Carrier", Japanese Patent Application No. 198,334/85, Sept. 7, 1985. A similar method uses a two-stage glutaraldehyde crosslinking procedure in which the crosslinking was interrupted after a period of time by washing away serum albumin that had not yet deposited on the silica. Subsequently, more glutaraldehyde was added to ensure that the remaining albumin was tightly crosslinked and permanently attached to the silica. The two stage process ensured that large clumps of support particles were not glued together. Such clumps disrupt flow through the column and degrade efficiency. See, e.g., R. A. Thompson et al, "Sorbents Obtained by Entrapment of Crosslinked Bovine Serum Albumin in Silica", Journal Chromatography, Vol. 465 (1989) pp. 263-270.

BSPR:

Reverse phase, cation exchange, and anion exchange organic resin or silica-based supports are sufficiently protein-adsorptive to be used, as is untreated silica. The preferred porous protein-adsorptive support is a porous silica support such as porous silica having a pore diameter of 30 to 300.Å, and a particle size of 1 to 500 micrometers; although, any protein-adsorptive support may be used. When a porous silica support is used it may be one already having an alkylsilane bonded to the surfaces thereof or it may be a dual zone or mixed phase material such as that shown in my U.S. Pat. No. 4,773,994, 4,778,600, 4,782,040, 4,950,634 and 4,950,635. In all instances, the coating of crosslinked protein covers the external surfaces of the porous silica support overcoating the alkyl or ketal-blocked-diol or fluoroalkyl or other external phase of the dual zone or mixed phase material.

CCOR:

210/198.2

DOCUMENT-IDENTIFIER: US 5512169 A
TITLE: Liquid column packing materials

BSPR:

Liquid column (LC) packing materials are usually porous materials which possess adsorptive or catalytic sites on the pore walls. They may be used as packings in columns or as loose material in vessels. LC packing materials typically are porous particles. However, they may instead be fibers or membranes. Porous membranes also provide filtration. When the pore size of the adsorptive or catalytic membrane excludes large proteins, then ultrafiltration is combined with adsorption or catalysis. Membranes may have small pores throughout their mass which exclude protein. Alternatively, the membrane may be a composite of sintered or adhered porous particles, in which case the pores between particles are large whereas the pores within the sintered or adhered particles are small. For example, Kontes Glass Co. markets a thick porous membrane of polyvinyl chloride upon whose large pore walls are attached small porous silica particles. The pores of the silica particles are much smaller than the pores upon whose walls the silica particles are attached.

BSPR:

A second approach to imparting a crosslinked protein coating onto packing materials employs simultaneous contact of glutaraldehyde with a concentrated solution of protein in an unbonded silica slurry in water. Such coated supports have high immobilized protein content and are useful for chromatography of dissolved protein. The object of this approach is to maximize the amount of immobilized protein short of creating an impermeable composite through which liquid could not readily flow. In this approach, the weak adsorption properties of the immobilized protein in the packing material

are useful. See, e.g., M. Tsuboi et al, "Chromatography Carrier", Japanese Patent Application No. 198,334/85, Sep. 7, 1985. A similar method uses a two-stage glutaraldehyde crosslinking procedure in which the crosslinking was interrupted after a period of time by washing away serum albumin that had not yet deposited on the silica. Subsequently, more glutaraldehyde was added to ensure that the remaining albumin was tightly crosslinked and permanently attached to the silica. The two stage process ensured that large clumps of support particles were not glued together. Such clumps disrupt flow through the column and degrade efficiency. See, e.g., R. A. Thompson et al, ". . . Sorbents Obtained by Entrapment of Crosslinked Bovine Serum Albumin in Silica", Journal Chromatography, Vol. 465 (1989) pp. 263-270.

BSPR:

Reverse phase, cation exchange, and anion exchange organic resin or silica-based supports are sufficiently protein-absorptive to be used, as is untreated silica. The preferred porous protein-adsorptive support is a porous silica support such as porous silica having a pore diameter of 30 to 300 .ANG., and a particle size of 1 to 500 micrometers; although, any protein-adsorptive support may be used. When a porous silica support is used it may be one already having an alkylsilane bonded to the surfaces thereof or it may be a dual zone or mixed phase material such as that shown in my U.S. Pat. Nos. 4,773,994, 4,778,600, 4,782,040, 4,950,634 and 4,950,635. In all instances, the coating of crosslinked protein covers the external surfaces of the porous silica support overcoating the alkyl or ketal-blocked-diol or fluoroalkyl or other external phase of the dual zone or mixed phase material.

CCOR:

210/198.2

DOCUMENT-IDENTIFIER: US 5470463 A

TITLE: Passivated porous supports and methods for the preparation and use of same

BSPR:

Several previous investigators have sought to passivate various microporous media including membranes and particulate chromatographic supports by applying thin surface coatings to inorganic or organic/polymeric substrates. For example, Steuck, in U.S. Pat. No. 4,618,533, discloses a porous polymeric membrane substrate fashioned from a thermoplastic organic polymer upon which a permanent coating is grafted and/or deposited on the entire membrane surface. The polymerization and crosslinking of the polymerizable monomer upon and within the porous membrane substrate is performed in such a way that a thin coating is deposited upon the entire surface of the porous membrane, including the inner pore walls. Significantly, the porous configurations of the coated, composite membrane structures claimed by Steuck are essentially identical to those of the corresponding uncoated porous membrane substrates, implying that the polymer of Steuck is applied as a thin surface layer or coating that does not interfere with the porosity or flow properties of his composite membranes. Moreover, Steuck does not disclose the concept of a "passivating layer" or the use of monomers capable of functioning as "passivating" monomers within the meaning of the present invention as discussed in more detail below.

BSPR:

While it has been discovered that this process of depositing protective polymer coatings upon the porous surfaces of mineral oxide (and particularly silica) matrices can significantly stabilize these materials by sharply reducing their

rates of chemical leaching, the approach has the important disadvantage of rendering the porous surfaces of the coated and protected matrices hydrophobic and thus prone to cause excessive non-specific binding of proteins by adsorption. (This is precisely the same problem noted above in connection with entirely polymeric porous support matrices.) However, this problem can be successfully addressed by the methods of the present invention in the same way as the non-specific binding of strictly polymeric support matrices can be reduced--i.e., by passivation in a process of oriented polymerization. More particularly, these composite chromatographic supports (i.e., supports comprised of mineral oxide substrates that have been stabilized by the application of thin protective polymer coatings) can be passivated against excessive non-specific binding by incorporating passivating ("neutralizing") monomers capable of associating with and consequently deactivating innate nonpolar hydrophobic groups exposed on the matrix surface. The passivating monomers useful in this embodiment of the present invention adsorb upon (and consequently cover) the hydrophobic groups on the surface by virtue of their containing long-chain saturated hydrocarbons, olefinic hydrocarbon groups, aromatic groups, or like hydrophobic domains that interact with and become appreciably bound to their hydrophobic counterparts on the matrix surface as a consequence of the hydrophobic-hydrophobic interaction existing between them. Typically, the present invention utilizes base matrices having the following characteristics: an initial average particle size ranging from about 5 to about 1000 microns; an initial porous volume ranging from about 0.2 to about 2 cm.³/gram; an initial surface area ranging from about 1 to about 800 m.²/gram; and an initial pore size ranging from about 50 to about 6000

angstroms. Preferably, the base matrix is characterized by: an initial average particle size ranging from about 10 to about 300 microns, although passivated supports having narrow particle size ranges, such as about 15-20, about 15-25, about 30-45, about 50-60, about 80-100, and about 100-300 microns, are most preferred. Preferred ranges for other characteristics include an initial porous volume ranging from about 0.8 to about 1.2 cm.³ /gram; an initial surface area ranging from about 10 to about 400 m.² /gram; and an initial pore size ranging from about 1000 to about 3000 angstroms. The density of the porous solid matrix obviously varies with its chemical nature, being higher for mineral oxide (e.g., silica) substrates and lower for polymeric ones (e.g., polystyrene).

DEPR:

It is thus demonstrated that the level of nonspecific adsorption for lysozyme

(a strong cationic protein) is high when the MAPTAC is absent. The

non-specific adsorption for silica with large surface areas (X 075, 100 m.² /g)

is higher (55 mg/ml of resin) than the non-specific adsorption for silica X

015 (25 m.² /g; 15 mg/ml of resin). A certain proportionality exists

between the surface area and the original level of non-specific absorptions.

The amount of MAPTAC to decrease the level of non-specific adsorption down to

zero is also proportional to the surface area available: 1.5% of MAPTAC is

necessary with silica X 015 (25 m.² /g) whereas at least 6% is necessary to

passivate silica X 075 (110 m.² /g).

DEPR:

The passivated resin is then recovered by filtration. The oil is eliminated

with an extensive washing with water containing 0.1-0.5% of a non-ionic

detergent and then stored in a saline buffer at neutral pH. The product resin

shows very similar ion-exchange characteristics as those described in Example 2. Additionally, its sensitivity in strong alkaline media is much improved as measured by its weight loss after one night of contact with 0.5M sodium hydroxide. The passivated resin of this example lost only about half as much weight as an anionic resin prepared from silica having an unprotected surface area.

DEPC:

Preparation of an Anion-Exchange Resin Using a Surface-Protected (i.e., Pre-coated) Silica Passivated Porous Support

DEPC:

Determination of Ion-Exchange and Protein Sorption Capacity of Preparation of Anion-Exchange Resins Based on Passivated Porous Silica Support of Different Surface Areas

CCOR:

210/198.2

DOCUMENT-IDENTIFIER: US 5445732 A

TITLE: Passivated porous polymer supports and methods for the preparation and use of same

BSPR:

Several previous investigators have sought to passivate various microporous media including membranes and particulate chromatographic supports by applying thin surface coatings to inorganic or organic/polymeric substrates. For example, Steuck, in U.S. Pat. No. 4,618,533, discloses a porous polymeric membrane substrate fashioned from a thermoplastic organic polymer upon which a permanent coating is grafted and/or deposited on the entire membrane surface. The polymerization and crosslinking of the polymerizable monomer upon and within the porous membrane substrate is performed in such a way that a thin coating is deposited upon the entire surface of the porous membrane, including the inner pore walls. Significantly, the porous configurations of the coated, composite membrane structures claimed by Steuck are essentially identical to those of the corresponding uncoated porous membrane substrates, implying that the polymer of Steuck is applied as a thin surface layer or coating that does not interfere with the porosity or flow properties of his composite membranes. Moreover, Steuck does not disclose the concept of a "passivating layer" or the use of monomers capable of functioning as "passivating" monomers within the meaning of the present invention as discussed in more detail below.

BSPR:

While it has been discovered that this process of depositing protective polymer coatings upon the porous surfaces of mineral oxide (and particularly silica) matrices can significantly stabilize these materials by sharply reducing their

rates of chemical leaching, the approach has the important disadvantage of rendering the porous surfaces of the coated and protected matrices hydrophobic and thus prone to cause excessive non-specific binding of proteins by adsorption. (This is precisely the same problem noted above in connection with entirely polymeric porous support matrices.) However, this problem can be successfully addressed by the methods of the present invention in the same way as the non-specific binding of strictly polymeric support matrices can be reduced--i.e., by passivation in a process of oriented polymerization. More particularly, these composite chromatographic supports (i.e., supports comprised of mineral oxide substrates that have been stabilized by the application of thin protective polymer coatings) can be passivated against excessive non-specific binding by incorporating passivating ("neutralizing") monomers capable of associating with and consequently deactivating innate non-polar hydrophobic groups exposed on the matrix surface. The passivating monomers useful in this embodiment of the present invention adsorb upon (and consequently cover) the hydrophobic groups on the surface by virtue of their containing long-chain saturated hydrocarbons, olefinic hydrocarbon groups, aromatic groups, or like hydrophobic domains that interact with and become appreciably bound to their hydrophobic counterparts on the matrix surface as a consequence of the hydrophobic-hydrophobic interaction existing between them. Typically, the present invention utilizes base matrices having the following characteristics: an initial average particle size ranging from about 5 to about 1000 microns; an initial porous volume ranging from about 0.2 to about 2 cm.³/gram; an initial surface area ranging from about 1 to about 800 m.²/gram; and an initial pore size ranging from about 50 to about 6000

angstroms. Preferably, the base matrix is characterized by: an initial average particle size ranging from about 10 to about 300 microns, although passivated supports having narrow particle size ranges, such as about 15-20, about 15-25, about 30-45, about 50-60, about 80-100, and about 100-300 microns, are most preferred. Preferred ranges for other characteristics include an initial porous volume ranging from about 0.8 to about 1.2 cm.³ /gram; an initial surface area ranging from about 10 to about 400 m.² /gram; and an initial pore size ranging from about 1000 to about 3000 angstroms. The density of the porous solid matrix obviously varies with its chemical nature, being higher for mineral oxide (e.g., silica) substrates and lower for polymeric ones (e.g., polystyrene).

DEPR:

It is thus demonstrated that the level of non-specific adsorption for lysozyme

(a strong cationic protein) is high when the MAPTAC is absent. The

non-specific adsorption for silica with large surface areas (X 075, 100 m.² /g)

is higher (55 mg/ml of resin) than the non-specific adsorption for silica X

015 (25 m.² /g; 15 mg/ml of resin). A certain proportionality exists

between the surface area and the original level of non-specific absorptions.

The amount of MAPTAC to decrease the level of non-specific adsorption down to

zero is also proportional to the surface area available: 1.5% of MAPTAC is

necessary with silica X 015 (25 m.² /g) whereas at least 6% is necessary to

passivate silica X 075 (100 m.² /g).

DEPR:

The passivated resin is then recovered by filtration. The oil is eliminated

with an extensive washing with water containing 0.1-0.5% of a non-ionic

detergent and then stored in a saline buffer at neutral pH. The product resin

shows very similar ion-exchange characteristics as those described in Example 2. Additionally, its sensitivity in strong alkaline media is much improved as measured by its weight loss after one night of contact with 0.5M sodium hydroxide. The passivated resin of this example lost only about half as much weight as an artionic resin prepared from silica having an unprotected surface area.

DEPL:

Preparation of an Anion-Exchange Resin Using a Surface-Protected (i.e., Pre-coated) Silica Passivated Porous Support

DEPL:

Determination of Ion-Exchange and Protein Sorption Capacity of Preparation of Artion-Exchange Resins Based on Passivated Porous Silica Support of Different Surface Areas

CCOR:

210/198.2

DOCUMENT-IDENTIFIER: US RE34910 E
TITLE: Carbon-clad zirconium oxide particles

BSPR:

Packing materials for high pressure liquid chromatography (HPLC) have also been based on carbon. For example, carbon-based supports useful for HPLC applications have included the following: graphitized carbon black (GCB), pyrocarbon reinforced GCB, and more recently, a porous graphitic carbon (PGC). PGC is prepared by filling the pores of a silica gel with a polymer comprising carbon, thermolyzing the polymer to produce a silica/carbon composite, dissolving out the silica to produce a porous carbon, and subjecting the porous carbon to graphitizing conditions.

BSPR:

For example, U.K. Patent Application No. 2,035,282 discloses a method for producing a porous carbon material suitable for chromatography or use as a catalyst support, which involves depositing carbon in the pores of a porous inorganic template material such as silica gel, porous glass, alumina or other porous refractory oxides having a surface area of at least 1 m.²/g, and thereafter removing the template material. O. Chiantore et al., Analytical Chemistry, 60, 638-642 (1988), disclose carbon sorbents which were prepared by pyrolysis of either phenol formaldehyde resin or saccharose on spheroidal silica gels coated with these materials. The pyrolysis is performed at 600.degree. C. for one hour in an inert atmosphere, and the silica is subsequently removed by boiling the material in an excess of a 10% NaOH solution for 30 minutes. Chiantore et al. conclude that, at the temperatures employed in their work, the carbonaceous polymer network that was formed still maintained some of the chemical features of the starting material

(page 641,
column 2). To obtain carbons where polar functional groups have
been
completely eliminated, the authors conclude that high
temperatures (greater
than 800.degree. C.) treatments under inert atmosphere are
necessary.

BSPR:

The present invention provides a composite support material which
is useful as
a stationary phase in liquid chromatography, particularly in
high-performance
liquid chromatography. The composite support material comprises
carbon-clad
particles of zirconium oxide (also referred to herein as
ZrO.sub.2, or as
"zirconia"). In order to facilitate packing of liquid
chromatography columns,
it is preferred that each individual unit of the present support
material be a
substantially spherical particle; thus, the preferred spherical
particles will
be referred to herein as "spherules." However, the present
invention is also
intended to provide support materials useful in low performance
chromatography,
fluidized beds, and general batch absorbers. There is no
requirement that the
present particles be substantially spherical in these
applications, where
irregularly shaped particles are typically utilized.

DEPR:

The present invention provides both a composite material useful
as a
chromatographic support, and a method for forming a
chromatographic support
material.

CCOR:

210/198.2

DOCUMENT-IDENTIFIER: US 5393430 A

TITLE: Passivated and stabilized porous mineral oxide supports and methods for the preparation and use of same

BSPR:

Several previous investigators have sought to passivate various microporous media including membranes and particulate chromatographic supports by applying thin surface coatings to inorganic or organic/polymeric substrates. For example, Steuck, in U.S. Pat. No. 4,618,533, discloses a porous polymeric membrane substrate fashioned from a thermoplastic organic polymer upon which a permanent coating is grafted and/or deposited on the entire membrane surface. The polymerization and crosslinking of the polymerizable monomer upon and within the porous membrane substrate is performed in such a way that a thin coating is deposited upon the entire surface of the porous membrane, including the inner pore walls. Significantly, the porous configurations of the coated, composite membrane structures claimed by Steuck are essentially identical to those of the corresponding uncoated porous membrane substrates, implying that the polymer of Steuck is applied as a thin surface layer or coating that does not interfere with the porosity or flow properties of his composite membranes. Moreover, Steuck does not disclose the concept of a "passivating layer" or the use of monomers capable of functioning as "passivating" monomers within the meaning of the present invention as discussed in more detail below.

BSPR:

While it has been discovered that this process of depositing protective polymer coatings upon the porous surfaces of mineral oxide (and particularly silica) matrices can significantly stabilize these materials by sharply reducing their

rates of chemical leaching, the approach has the important disadvantage of rendering the porous surfaces of the coated and protected matrices hydrophobic and thus prone to cause excessive non-specific binding of proteins by adsorption. (This is precisely the same problem noted above in connection with entirely polymeric porous support matrices.) However, this problem can be successfully addressed by the methods of the present invention in the same way as the non-specific binding of strictly polymeric support matrices can be reduced--i.e., by passivation in a process of oriented polymerization. More particularly, these composite chromatographic supports (i.e., supports comprised of mineral oxide substrates that have been stabilized by the application of thin protective polymer coatings) can be passivated against excessive non-specific binding by incorporating passivating ("neutralizing") monomers capable of associating with and consequently deactivating innate non-polar hydrophobic groups exposed on the matrix surface. The passivating monomers useful in this embodiment of the present invention adsorb upon (and consequently cover) the hydrophobic groups on the surface by virtue of their containing long-chain saturated hydrocarbons, olefinic hydrocarbon groups, aromatic groups, or like hydrophobic domains that interact with and become appreciably bound to their hydrophobic counterparts on the matrix surface as a consequence of the hydrophobic-hydrophobic interaction existing between them. Typically, the present invention utilizes base matrices having the following characteristics: an initial average particle size ranging from about 5 to about 1000 microns; an initial porous volume ranging from about 0.2 to about 2 cm.³/gram; an initial surface area ranging from about 1 to about 800 m.²/gram; and an initial pore size ranging from about 50 to about 6000

angstroms. Preferably, the base matrix is characterized by: an initial average particle size ranging from about 10 to about 300 microns, although passivated supports having narrow particle size ranges, such as about 15-20, about 15-25, about 30-45, about 50-60, about 80-100, and about 100-300 microns, are most preferred. Preferred ranges for other characteristics include an initial porous volume ranging from about 0.8 to about 1.2 cm.³ /gram; an initial surface area ranging from about 10 to about 400 m.² /gram; and an initial pore size ranging from about 1000 to about 3000 angstroms. The density of the porous solid matrix obviously varies with its chemical nature, being higher for mineral oxide (e.g., silica) substrates and lower for polymeric ones (e.g., polystyrene).

DEPR:

It is thus demonstrated that the level of non-specific adsorption for lysozyme

(a strong cationic protein) is high when the MAPTAC is absent.

The

non-specific adsorption for silica with large surface areas (X 075, 100 m.² /g)

is higher (55 mg/ml of resin) than the non-specific adsorption for silica X

015 (25 m.² /g; 15 mg/ml of resin). A certain proportionality exists

between the surface area and the original level of non-specific absorptions.

The amount of MAPTAC to decrease the level of non-specific adsorption down to

zero is also proportional to the surface area available: 1.5% of MAPTAC is

necessary with silica X 015 (25 m.² /g) whereas at least 6% is necessary to

passivate silica X 075 (100 m.² /g).

DEPR:

The passivated resin is then recovered by filtration. The oil is eliminated

with an extensive washing with water containing 0.1-0.5% of a non-ionic

detergent and then stored in a saline buffer at neutral pH. The product resin

shows very similar ion-exchange characteristics as those described in Example 2. Additionally, its sensitivity in strong alkaline media is much improved as measured by its weight loss after one night of contact with 0.5M sodium hydroxide. The passivated resin of this example lost only about half as much weight as an artionic resin prepared from silica having an unprotected surface area.

DEPL:

Preparation of an Anion-Exchange Resin Using a Surface-Protected (i.e., Pre-coated) Silica Passivated Porous Support

DEPL:

Determination of Ion-Exchange and Protein Sorption Capacity of Preparation of Anion-Exchange Resins Based on Passivated Porous Silica Support of Different Surface Areas

CCXR:

210/198.2

DOCUMENT-IDENTIFIER: US 5346619 A
TITLE: Carbon-clad zirconium oxide particles

BSPR:

Packing materials for high pressure liquid chromatography (HPLC) have also been based on carbon. For example, carbon-based supports useful for HPLC

applications have included the following: graphitized carbon black (GCB), pyrocarbon reinforced GCB, and more recently, a porous graphitic carbon (PGC).

PGC is prepared by filling the pores of a silica gel with a polymer comprising carbon, thermolyzing the polymer to produce a silica/carbon composite,

dissolving out the silica to produce a porous carbon, and subjecting the porous carbon to graphitizing conditions.

BSPR:

For example, U.K. Patent Application No. 2,035,282 discloses a method for producing a porous carbon material suitable for chromatography or use as a catalyst support, which involves depositing carbon in the pores of a porous inorganic template material such as silica gel, porous glass, alumina or other porous refractory oxides having a surface area of at least 1 m.²/g, and thereafter removing the template material. O. Chiantore et al., Analytical

Chemistry, 60, 638-642 (1988), disclose carbon sorbents which were prepared by pyrolysis of either phenol formaldehyde resin or saccharose on spheroidal

silica gels coated with these materials. The pyrolysis is performed at

600.degree. C. for one hour in an inert atmosphere, and the silica is subsequently removed by boiling the material in an excess of a 10% NaOH

solution for 30 minutes. Chiantore et al. conclude that, at the temperatures

employed in their work, the carbonaceous polymer network that was formed still

maintained some of the chemical features of the starting material

(page 641,
column 2). To obtain carbons where polar functional groups have
been
completely eliminated, the authors conclude that high
temperatures (greater
than 800.degree. C.) treatments under inert atmosphere are
necessary.

BSPR:

The present invention provides a composite support material which
is useful as
a stationary phase in liquid chromatography, particularly in
high-performance
liquid chromatography. The composite support material comprises
carbon-clad
particles of zirconium oxide (also referred to herein as
ZrO.sub.2, or as
"zirconia"). In order to facilitate packing of liquid
chromatography columns,
it is preferred that each individual unit of the present support
material be a
substantially spherical particle; thus, the preferred spherical
particles will
be referred to herein as "spherules". However, the present
invention is also
intended to provide support materials useful in low performance
chromatography,
fluidized beds, and general batch absorbers. There is no
requirement that the
present particles be substantially spherical in these
applications, where
irregularly shaped particles are typically utilized.

DEPR:

The present invention provides both a composite material useful
as a
chromatographic support, and a method for forming a
chromatographic support
material.

CCOR:

210/198.2

DOCUMENT-IDENTIFIER: US 5271833 A

TITLE: Polymer-coated carbon-clad inorganic oxide particles

BSPR:

Packing materials for high pressure liquid chromatography (HPLC) have also been based on carbon. For example, carbon-based supports useful for HPLC

applications have included the following: graphitized carbon black (GCB),

pyrocarbon reinforced GCB, and more recently, a porous graphitic carbon (PGC).

PGC is prepared by filling the pores of a silica gel with a polymer comprising

carbon, thermolyzing the polymer to produce a silica/carbon composite,

dissolving out the silica to produce a porous carbon, and subjecting the porous carbon to graphitizing conditions.

BSPR:

For example, U.K. Patent Application No. 2,035,282 discloses a method for

producing a porous carbon material suitable for chromatography or use as a

catalyst support, which involves depositing carbon in the pores of a porous

inorganic template material such as silica gel, porous glass, alumina or other

porous refractory oxides having a surface area of at least 1 m.²/g, and

thereafter removing the template material. O. Chiantore et al., Analytical

Chemistry, 60, 638-642 (1988), disclose carbon sorbents which were prepared by

pyrolysis of either phenol formaldehyde resin or saccharose on spheroidal

silica gels coated with these materials. The pyrolysis is performed at

600.degree. C. for one hour in an inert atmosphere, and the silica is

subsequently removed by boiling the material in an excess of a 10% NaOH

solution for 30 minutes. Chiantore et al. conclude that, at the temperatures

employed in their work, the carbonaceous polymer network that was formed still

maintained some of the chemical features of the starting material

(page 641,
column 2). To obtain carbons where polar functional groups have
been
completely eliminated, the authors conclude that high
temperatures (greater
than 800.degree. C.) treatments under inert atmosphere are
necessary.

BSPR:

The present invention provides a composite support material which
is useful as
a stationary phase in liquid chromatography, particularly in
high-performance
liquid chromatography. The composite support material comprises
carbon-clad
particles of zirconium oxide (also referred to herein as
ZrO₂, or as
"zirconia"). In order to facilitate packing of liquid
chromatography columns,
it is preferred that each individual unit of the present support
material be a
substantially spherical particle; thus, the preferred spherical
particles will
be referred to herein as "spherules." However, the present
invention is also
intended to provide support materials useful in low performance
chromatography,
fluidized beds, and general batch absorbers. There is no
requirement that the
present particles be substantially spherical in these
applications, where
irregularly shaped particles are typically utilized.

DEPR:

The present invention provides both a composite material useful
as a
chromatographic support, and a method for forming a
chromatographic support
material.

CCOR:

210/198.2

DOCUMENT-IDENTIFIER: US 5270280 A

TITLE: Packing material for liquid chromatography and method of manufacturing thereof

BSPR:

As a packing material for liquid chromatography, there are conventionally used a chemically bonded type of packing material based on silica gel and a packing material based on synthetic resin. The silica gel-based packing material is relatively strong in mechanical strength and is small in swelling/shrinking characteristics against various organic solvents. Therefore, it has a high resolving power and is superior in exchangeability of eluent for analysis.

BSPR:

The granulated particles obtained are composite bodies comprising carbon black and binder. In the first aspect of this invention, the composite bodies are heated to 800.degree. C. to 3000.degree. C. for graphitization (carbonization) of the binder, and consequently a packing material for liquid chromatography of this invention is obtained. If the graphitization (carbonization) temperature is below 800.degree. C., the graphitization (carbonization) of the binder is not sufficient, resulting in an insufficient strength of the packing material. If the graphitization (carbonization) temperature is above 3000.degree. C., it is not preferable in that the graphitization (carbonization) yield is remarkably lowered and that the strength of the packing material is decreased. This graphitizing (carbonizing) treatment is carried out in an inert gas or under vacuum. It is preferable, prior to the graphitizing (carbonizing) treatment, to first heat the granulated particles to about 150.degree. C. to evaporate the organic binding agent in the composite particles and then to heat them to about

500.degree. C. to
harden and infusibilize the binder.

CCXR:

210/198.2

DOCUMENT-IDENTIFIER: US 5268097 A

TITLE: Passivated and stabilized porous mineral oxide supports and method for the preparation and use of same

BSPR:

Several previous investigators have sought to passivate various microporous media including membranes and particulate chromatographic supports by applying thin surface coatings to inorganic or organic/polymeric substrates. For example, Steuck, in U.S. Pat. No. 4,618,533, discloses a porous polymeric membrane substrate fashioned from a thermoplastic organic polymer upon which a permanent coating is grafted and/or deposited on the entire membrane surface. The polymerization and crosslinking of the polymerizable monomer upon and within the porous membrane substrate is performed in such a way that a thin coating is deposited upon the entire surface of the porous membrane, including the inner pore walls. Significantly, the porous configurations of the coated, composite membrane structures claimed by Steuck are essentially identical to those of the corresponding uncoated porous membrane substrates, implying that the polymer of Steuck is applied as a thin surface layer or coating that does not interfere with the porosity or flow properties of his composite membranes. Moreover, Steuck does not disclose the concept of a "passivating layer" or the use of monomers capable of functioning as "passivating" monomers within the meaning of the present invention as discussed in more detail below.

BSPR:

While it has been discovered that this process of depositing protective polymer coatings upon the porous surfaces of mineral oxide (and particularly silica) matrices can significantly stabilize these materials by sharply reducing their

rates of chemical leaching, the approach has the important disadvantage of rendering the porous surfaces of the coated and protected matrices hydrophobic and thus prone to cause excessive non-specific binding of proteins by adsorption. (This is precisely the same problem noted above in connection with entirely polymeric porous support matrices.) However, this problem can be successfully addressed by the methods of the present invention in the same way as the non-specific binding of strictly polymeric support matrices can be reduced--i.e., by passivation in a process of oriented polymerization. More particularly, these composite chromatographic supports (i.e., supports comprised of mineral oxide substrates that have been stabilized by the application of thin protective polymer coatings) can be passivated against excessive non-specific binding by incorporating passivating ("neutralizing") monomers capable of associating with and consequently deactivating innate non-polar hydrophobic groups exposed on the matrix surface. The passivating monomers useful in this embodiment of the present invention adsorb upon (and consequently cover) the hydrophobic groups on the surface by virtue of their containing long-chain saturated hydrocarbons, olefinic hydrocarbon groups, aromatic groups, or like hydrophobic domains that interact with and become appreciably bound to their hydrophobic counterparts on the matrix surface as a consequence of the hydrophobic-hydrophobic interaction existing between them. Typically, the present invention utilizes base matrices having the following characteristics: an initial average particle size ranging from about 5 to about 1000 microns; an initial porous volume ranging from about 0.2 to about 2 cm.³ /gram; an initial surface area ranging from about 1 to about 800 m.² /gram; and an initial pore size ranging from about 50 to about 6000

angstroms. Preferably, the base matrix is characterized by: an initial average particle size ranging from about 10 to about 300 microns, although passivated supports having narrow particle size ranges, such as about 15-20, about 15-25, about 30-45, about 50-60, about 80-100, and about 100-300 microns, are most preferred. Preferred ranges for other characteristics include an initial porous volume ranging from about 0.8 to about 1.2 cm.³ /gram; an initial surface area ranging from about 10 to about 400 m.² /gram; and an initial pore size ranging from about 1000 to about 3000 angstroms. The density of the porous solid matrix obviously varies with its chemical nature, being higher for mineral oxide (e.g., silica) substrates and lower for polymeric ones (e.g., polystyrene).

DEPR:

It is thus demonstrated that the level of non-specific adsorption for lysozyme

(a strong cationic protein) is high when the MAPTAC is absent. The

non-specific adsorption for silica with large surface areas (X 075, 100 m.² /g)

is higher (55 mg/ml of resin) than the non-specific adsorption for silica X

015 (25 m.² /g; 15 mg/ml of resin). A certain proportionality exists

between the surface area and the original level of non-specific absorptions.

The amount of MAPTAC to decrease the level of non-specific adsorption down to

zero is also proportional to the surface area available: 1.5% of MAPTAC is

necessary with silica X 015 (25 m.² /g) whereas at least 6% is necessary to

passivate silica X 075 (100 m.² /g).

DEPR:

The passivated resin is then recovered by filtration. The oil is eliminated

with an extensive washing with water containing 0.1-0.5% of a non-ionic

detergent and then stored in a saline buffer at neutral pH. The product resin

shows very similar ion-exchange characteristics as those described in Example 2. Additionally, its sensitivity in strong alkaline media is much improved as measured by its weight loss after one night of contact with 0.5M sodium hydroxide. The passivated resin of this example lost only about half as much weight as an artionic resin prepared from silica having an unprotected surface area.

DEPC:

Preparation of an Anion-Exchange Resin Using a Surface-Protected (i.e., Pre-coated) Silica Passivated Porous Support

DEPC:

Determination of Ion-Exchange and Protein Sorption Capacity of Preparation of Anion-Exchange Resins Based on Passivated Porous Silica Support of Different Surface Areas

CCOR:

210/198.2

6

DOCUMENT-IDENTIFIER: US 5182016 A
TITLE: Polymer-coated carbon-clad inorganic oxide particles

BSPR:

Packing materials for high pressure liquid chromatography (HPLC) have also been based on carbon. For example, carbon-based supports useful for HPLC

applications have included the following: graphitized carbon black (GCB), pyrocarbon reinforced GCB, and more recently, a porous graphitic carbon (PGC).

PGC is prepared by filling the pores of a silica gel with a polymer comprising carbon, thermolyzing the polymer to produce a silica/carbon composite,

dissolving out the silica to produce a porous carbon, and subjecting the porous carbon to graphitizing conditions.

BSPR:

For example, U.K. Patent Application No. 2,035,282 discloses a method for producing a porous carbon material suitable for chromatography or use as a

catalyst support, which involves depositing carbon in the pores of a porous

inorganic template material such as silica gel, porous glass, alumina or other

porous refractory oxides having a surface area of at least 1 m.^{sup.2} /g, and

thereafter removing the template material. O. Chiantore et al., Analytical

Chemistry, 60, 638-642 (1988), disclose carbon sorbents which were prepared by

pyrolysis of either phenol formaldehyde resin or saccharose on spheroidal

silica gels coated with these materials. The pyrolysis is performed at

600.degree. C. for one hour in an inert atmosphere, and the silica is

subsequently removed by boiling the material in an excess of a 10% NaOH

solution for 30 minutes. Chiantore et al. conclude that, at the temperatures

employed in their work, the carbonaceous polymer network that was formed still

maintained some of the chemical features of the starting material

(page 641,
column 2). To obtain carbons where polar functional groups have
been
completely eliminated, the authors conclude that high
temperatures (greater
than 800.degree. C.) treatments under inert atmosphere are
necessary.

BSPR:

The present invention provides a composite support material which
is useful as
a stationary phase in liquid chromatography, particularly in
high-performance
liquid chromatography. The composite support material comprises
carbon-clad
particles of zirconium oxide (also referred to herein as
ZrO.sub.2, or as
"zirconia"). In order to facilitate packing of liquid
chromatography columns,
it is preferred that each individual unit of the present support
material be a
substantially spherical particle; thus, the preferred spherical
particles will
be referred to herein as "spherules." However, the present
invention is also
intended to provide support materials useful in low performance
chromatography,
fluidized beds, and general batch absorbers. There is no
requirement that the
present particles be substantially spherical in these
applications, where
irregularly shaped particles are typically utilized.

DEPR:

The present invention provides both a composite material useful
as a
chromatographic support, and a method for forming a
chromatographic support
material.

CCOR:

210/198.2

DOCUMENT-IDENTIFIER: US 5135649 A

TITLE: Column packing material with both hydrophobic and hydrophilic groups and process for production thereof

DEPR:

As the porous support to be used in the present invention, there can be employed, for example, silica gel, alumina, glass beads (e.g., porous glass), zeolite, hydroxyapatite, or graphite; namely, any powder generally employed as the support for chromatography. Also, a composite powder, such as a powder coated with a fine inorganic powder, for example, silica gel, titanium oxide or hydroxyapatite, on the surface of a synthetic resin such as polyamide, acrylic resin, or polyvinyl alcohol, can be employed.

CLPR:

3. A column packing material as claimed in claim 1, wherein the porous support is a composite powder comprising a synthetic resin coated with a fine inorganic powder.

CCOR:

210/198.2

DOCUMENT-IDENTIFIER: US 5108597 A
TITLE: Carbon-clad zirconium oxide particles

BSPR:

Packing materials for high pressure liquid chromatography (HPLC) have also been based on carbon. For example, carbon-based supports useful for HPLC

applications have included the following: graphitized carbon black (GCB), pyrocarbon reinforced GCB, and more recently, a porous graphitic carbon (PGC).

PGC is prepared by filling the pores of a silica gel with a polymer comprising carbon, thermolyzing the polymer to produce a silica/carbon composite,

dissolving out the silica to produce a porous carbon, and subjecting the porous carbon to graphitizing conditions.

BSPR:

For example, U.K. Patent Application No. 2,035,282 discloses a method for producing a porous carbon material suitable for chromatography or use as a

catalyst support, which involves depositing carbon in the pores of a porous inorganic template material such as silica gel, porous glass, alumina or other

porous refractory oxides having a surface area of at least 1 m.²/g, and

thereafter removing the template material. O. Chiantore et al., Analytical

Chemistry, 60, 638-642 (1988), disclose carbon sorbents which were prepared by

pyrolysis of either phenol formaldehyde resin or saccharose on spheroidal

silica gels coated with these materials. The pyrolysis is performed at

600.degree. C. for one hour in an inert atmosphere, and the silica is

subsequently removed by boiling the material in an excess of a 10% NaOH

solution for 30 minutes. Chiantore et al. conclude that, at the temperatures

employed in their work, the carbonaceous polymer network that was formed still

maintained some of the chemical features of the starting material

(page 641,
column 2). To obtain carbons where polar functional groups have
been
completely eliminated, the authors conclude that high
temperatures (greater
than 800.degree. C) treatments under inert atmosphere are
necessary.

BSPR:

The present invention provides a composite support material which
is useful as
a stationary phase in liquid chromatography, particularly in
high-performance
liquid chromatography. The composite support material comprises
carbon-clad
particles of zirconium oxide (also referred to herein as
ZrO.sub.2, or as
"zirconia"). In order to facilitate packing of liquid
chromatography columns,
it is preferred that each individual unit of the present support
material be a
substantially spherical particle; thus, the preferred spherical
particles will
be referred to herein as "spherules." However, the present
invention is also
intended to provide support materials useful in low performance
chromatography,
fluidized beds, and general batch absorbers. There is no
requirement that the
present particles be substantially spherical in these
applications, where
irregularly shaped particles are typically utilized.

DEPR:

The present invention provides both a composite material useful
as a
chromatographic support, and a method for forming a
chromatographic support
material.

CCOR:

210/198.2

DOCUMENT-IDENTIFIER: US 4314823 A
TITLE: Combination apparatus and method for chromatographic separation and quantitative analysis of multiple ionic species

DEPR:

As set out above, the ion exclusion resin for anion separation is preferably in the hydrogen ion form. This permits conversion of the weak acids to their unionized molecular form. In addition, it causes stripping of cations which could provide interference background in conductivity cell 18. However, if desired for specialty applications, non-ionic resin may be employed, so long as the counterions in the sample are preconverted to acid form for the separation of anions or base form for the separation of cations. This is preferably performed by using a strong acid or strong base eluent. Suitable non-ionic resins are so-called silica based reverse phase resins. Specific suitable ones are sold under the trade designation .mu.-BondaPak C-18 by Waters Associates.

DEPR:

The use of a concentrator column in the present system increases its versatility. Thus, it permits the concentration of ionic species in the absence of its accompanying solution so that upon removal with the developing reagent, there is a minimum disturbance of the equilibrium of the separation column. In addition, if desired, multiple injections of the same sample may be made to supply multiple quantities of ionic species on the concentrator column to be resolved as a composite sample on ion chromatography column 53. This is particularly useful for trace quantities of an ionic species which may be difficult to resolve using a single sample. Also, by use of multiple concentrator columns (not shown) and/or appropriate adjustment of the valving,

it is possible to resolve predetermined highly specific peaks by repeated runs of preselected ionic species. Thus, although the system has been described in terms of using ion chromatography to resolve only the strong acids or bases which pass through the void volume peak by the ion chromatography stage, it should be understood that the weak ionic species which are at least partially resolved during ion exclusion on column 37 may be further resolved by ion chromatography in column 50. This may be done by loading such ionic species on the illustrated single concentrator column or by the use of multiple concentrator columns (not shown) in parallel and by appropriate adjustment in the valving. Thus, since ion exclusion and ion chromatography resolve the ionic species by different mechanisms, it may be possible that two weak acid or base ionic species may be difficult to fully resolve by ion exclusion alone but may be fully resolved by a combination of ion exclusion chromatography on separation column 37 and ion chromatography on separation column 53. One or more concentrator columns 49 facilitate this viewing or preselected peaks of ionic species.

CCXR:

210/198.2

Printed by EAST

UserID: ETherkorn

Computer: WS08186

Date: 08/03/2000

Time: 09:53

Document Listing

Document	Image pages	Text pages	Error pages
US 6063283 A	0	1	0
US 5738783 A	0	1	0
US 5135649 A	0	1	0
Total	0	3	0

DOCUMENT-IDENTIFIER: US 6063283 A

TITLE: Method for analyzing a sample by using a liquid chromatograph

DEPR:

In the present invention, porous media commonly used in liquid chromatography

such as silica gel, alumina, porous glass beads, zeolite, hydroxyapatite and

graphite may be used as the packing material of the column 40.

Alternatively,

it is possible to use composite powders formed of a resin core covered by

inorganic powders such as silica gel, titanium dioxide,

hydroxyapatite, and the

like. One may use materials such as polyamides, acrylic resins, polyvinyl

alcohols, and the like, for the resin core.

CCOR:

210/656

CCXR:

210/198.2

DOCUMENT-IDENTIFIER: US 5738783 A

TITLE: Liquid chromatograph having a micro- and semi-micro column

DEPR:

In the present invention, porous media commonly used in liquid chromatography

such as silica gel, alumina, porous glass beads, zeolite, hydroxyapatite and

graphite may be used as the packing material of the column 40.

Alternatively,

it is possible to use composite powders formed of a resin core covered by

inorganic powders such as silica gel, titanium dioxide, hydroxyapatite, and the

like. One may use materials such as polyamides, acrylic resins, polyvinyl

alcohols, and the like, for the resin core.

CCOR:

210/198.2

CCXR:

210/502.1

CCXR:

210/635

CCXR:

210/656

DOCUMENT-IDENTIFIER: US 5135649 A
TITLE: Column packing material with both hydrophobic and hydrophilic groups and process for production thereof

DEPR:

As the porous support to be used in the present invention, there can be employed, for example, silica gel, alumina, glass beads (e.g., porous glass), zeolite, hydroxyapatite, or graphite; namely, any powder generally employed as the support for chromatography. Also, a composite powder, such as a powder coated with a fine inorganic powder, for example, silica gel, titanium oxide or hydroxyapatite, on the surface of a synthetic resin such as polyamide, acrylic resin, or polyvinyl alcohol, can be employed.

CCOR:

210/198.2

CCXR:

210/502.1

CCXR:

210/635

CCXR:

210/656

Printed by EAST

UserID: ETherkorn

Computer: WS08186

Date: 08/03/2000

Time: 09:55

Document Listing

Document	Image pages	Text pages	Error pages
US 5738783 A	0	1	0
US 5135649 A	0	1	0
Total	0	2	0

DOCUMENT-IDENTIFIER: US 5738783 A

TITLE: Liquid chromatograph having a micro- and semi-micro column

DEPR:

In the present invention, porous media commonly used in liquid chromatography

such as silica gel, alumina, porous glass beads, zeolite, hydroxyapatite and

graphite may be used as the packing material of the column 40.

Alternatively,

it is possible to use composite powders formed of a resin core covered by

inorganic powders such as silica gel, titanium dioxide, hydroxyapatite, and the

like. One may use materials such as polyamides, acrylic resins, polyvinyl

alcohols, and the like, for the resin core.

CCXR:

210/635

DOCUMENT-IDENTIFIER: US 5135649 A

TITLE: Column packing material with both hydrophobic and hydrophilic groups and process for production thereof

DEPR:

As the porous support to be used in the present invention, there can be employed, for example, silica gel, alumina, glass beads (e.g., porous glass), zeolite, hydroxyapatite, or graphite; namely, any powder generally employed as the support for chromatography. Also, a composite powder, such as a powder coated with a fine inorganic powder, for example, silica gel, titanium oxide or hydroxyapatite, on the surface of a synthetic resin such as polyamide, acrylic resin, or polyvinyl alcohol, can be employed.

CCXR:

210/635

Printed by EAST

UserID: ETherkorn

Computer: WS08186

Date: 08/03/2000

Time: 09:57

Document Listing

Document	Image pages	Text pages	Error pages
US 5738783 A	0	1	0
US 5135649 A	0	1	0
Total	0	2	0

DOCUMENT-IDENTIFIER: US 5738783 A

TITLE: Liquid chromatograph having a micro- and semi-micro column

DEPR:

In the present invention, porous media commonly used in liquid chromatography

such as silica gel, alumina, porous glass beads, zeolite, hydroxyapatite and

graphite may be used as the packing material of the column 40.

Alternatively,

it is possible to use composite powders formed of a resin core covered by

inorganic powders such as silica gel, titanium dioxide, hydroxyapatite, and the

like. One may use materials such as polyamides, acrylic resins, polyvinyl

alcohols, and the like, for the resin core.

CCXR:

210/502.1

DOCUMENT-IDENTIFIER: US 5135649 A

TITLE: Column packing material with both hydrophobic and hydrophilic groups and process for production thereof

DEPR:

As the porous support to be used in the present invention, there can be employed, for example, silica gel, alumina, glass beads (e.g., porous glass), zeolite, hydroxyapatite, or graphite; namely, any powder generally employed as the support for chromatography. Also, a composite powder, such as a powder coated with a fine inorganic powder, for example, silica gel, titanium oxide or hydroxyapatite, on the surface of a synthetic resin such as polyamide, acrylic resin, or polyvinyl alcohol, can be employed.

CCXR:

210/502.1



Printed by EAST

UserID: ETTherkorn

Computer: WS08186

Date: 08/03/2000

Time: 10:02

Document Listing

Document	Image pages	Text pages	Error pages
US 5997887 A	0	3	0
US 5393362 A	0	1	0
US 5238810 A	0	1	0
US 5190795 A	0	1	0
Total	0	6	0

DOCUMENT-IDENTIFIER: US 5997887 A
TITLE: Skin care compositions and method of improving skin appearance

BSPR:

The reflective particulate material preferably comprises particles of inorganic material comprising TiO_2 , ZnO, ZrO_2 and combinations thereof, more preferably TiO_2 , ZnO and combinations thereof (combinations are intended to include particles which comprise one or more of these materials, as well as mixtures of these particulate materials) and most preferably, the particles consist essentially of TiO_2 . The particulate material may be a composite, e.g., deposited on a core or mixed with other materials such as, but not limited to, silica, silicone resin, mica, and nylon.

DEPR:

The Coverage Index values of the compositions in Examples 1-3 are obtained by the following protocol: Using a collagen film having an exposed surface area of about 7 cm² (such as IMS #1192 or equivalent available from IMS Inc. Milford Conn.) mounted in a suitable holder, apply 40 microliters (using a Microman M50 pipette) of product and spread evenly by hand on the film surface using 10 finger rotations. Optionally, the sample is mounted on a Zeiss SV-11 microscope (or equivalent) equipped with a 1X lens (the microscope is useful for enlarging the image which the camera is capturing; the effective magnification of this system is about 5 microns/pixel). A mounting template can optionally be employed to aid in repositioning the sample for multiple measurements. The SV-11 should be set up so that maximum light is being transmitted to the camera (e.g., a Sony 760-MD CCD 3 Camera). To insure proper positioning and a clear image, the equipment is set in the following manner.

Camera controls are set so that the Gamma and Linear matrix switches are off.
The camera control box settings are further defined as follows:
Gain=0,
White/Black balance on auto, iris- auto, mode-camera, detail-12 o'clock position, phase-0 degrees, SC-3 o'clock position, H-12 o'clock position, Color temp -3200 K, shutter off. The camera should be allowed to warm up for 15 minutes before adjusting white and black balance. Press the button labeled "white" to adjust the white balance, and adjust black balance by pulling the black adjustment rod and pushing the button that says black.
Computer cables are connected to the RGB1 and Composite Sync.ports on the camera.
The microscope Iris is set to completely open and a frosted glass plate is positioned in the microscope base for a uniformly lit field. A clear glass plate may optionally be used to adjust the sample height. Open the Optimas 5.2 program on the computer. Use a sample cup which is partially covered with black tape in the light path to adjust gain and offset (brightness). The reflecting mirror at the base of the microscope is set for maximum reflection into the microscope. The mean of the light source should be 245 to 254.5. The STD Deviation should be less than 3. If the mean is out of specifications check the light bulb alignment and mirror adjustment.

DEPR:

For measuring light transmission by the test product, a piece of collagen film, having a surface area of about 7 cm.², is first pre-hydrated with distilled water to insure flexibility. 40 microliters of test product is then dispensed on the film (e.g., using a Microman M50 pipette or equivalent), and spread evenly over its surface to produce an even film covering the surface of the collagen (generally by lightly spreading the material by applying 10 rotations of the finger, wearing a clean, latex finger cot, to the

material). After
waiting for a period of 5 minutes, the sample is mounted on the
microscope
base. Light transmission measurements through the film and
product are then
taken in the manner described for the control. Least significant
differences
can be performed on the data using Fischer's LSD method. The
Coverage Index is
calculated as follows: ##EQU3##

DOCUMENT-IDENTIFIER: US 5393362 A
TITLE: Method for improving adhesion to metal

BSPR:

Composites that can be used include conventional composites containing an adhesive resin as described above and a filler such as silica, glass, and the like. Commercially available composites include dental restorative composites such as P-50.TM. and P-30.TM. dental restoratives (3M).

DEPR:

Various porcelain-fusing metal surfaces were prepared using the general procedure of EXAMPLE 1 with the following exceptions: in Step 1, the metal was dry polished; in Step 2, the surface was sandblasted for 1 minute; in Step 3, the samples were rinsed with distilled water only; an additional step (Step 3a) was added between Step 3 and Step 4, which consisted of heating the samples in a Ney STAR FIRE.TM. high performance programmable porcelain furnace (#949-14-002, J. M. Ney Company, Bloomfield, Conn.) at a particular furnace temperature for the respective metal substrate as follows: REXILLIUM.TM. III (Jeneric Industries, Inc., Wallingford, Conn.) and stainless steel 316 (3M Unitek) were heated at 1051.degree. C., RX NATURELLE.TM. (Jeneric Industries) was heated at 537.degree. C. and RX SP CG.TM. (Jeneric Industries) was heated at 1093.degree. C.; in Step 4, the organosilicon compound was applied by placing one drop of the A-174 solution on the surface of the metal disk with a plastic transfer pipet; and in Step 5, the samples were heated in the porcelain furnace described in Step 3a at 500.degree. C. for 10 minutes.

DOCUMENT-IDENTIFIER: US 5238810 A

TITLE: Laser magnetic immunoassay method and apparatus thereof

DEPR:

The material of the non-magnetic carrier particles may be for example, acrylic polymer resin or polystyrene resin microspheres, or non-magnetic alumina or silica colloidal particles. Whatever the choice of material, the density of the non-magnetic carrier particles should be greater than that of the magnetic particles because during the centrifugation procedure, the non-magnetic carrier particles are caused to precipitate and the unreacted magnetic particle labeled antibody reagent is discarded as the supernatant. This desired increased density may be achieved by employing a core of some dense, non-ferrous metal such as lead in the form of an organic or inorganic composite material.

DEPR:

Subsequently, as shown in FIG. 25(g), an alcohol such as methanol or ethanol was added to the well X with a micro-syringe 8e or pipette to dissolve the Novolak resin film to fluidize the specimen. The addition of alcohol was performed preferably by firstly removing most of the liquid in the well with a micro-syringe, adding an alcohol and then adding water. In the drawing, decomposed support is schematically indicated by a symbol (.DELTA.) and reference numeral 81a. Actually, a part of the support 81 is dispersed as solid. Thus, the antigen-antibody complex 80-82-82-84 containing micro-particles of the magnetic substance 84 floated in the well X. In this case, although a heater 85 is described in the drawing, it was not used (unnecessary).

DOCUMENT-IDENTIFIER: US 5190795 A
TITLE: Method for improving adhesion to metal

BSPR:

Composites that can be used include conventional composites containing an adhesive resin as described above and a filler such as silica, glass, and the like. Commercially available composites include dental restorative composites such as P-50.TM. and P-30.TM. dental restoratives (3M).

DEPR:

Various porcelain-fusing metal surfaces were prepared using the general procedure of EXAMPLE 1 with the following exceptions: in Step 1, the metal was dry polished; in Step 2, the surface was sandblasted for 1 minute; in Step 3, the samples were rinsed with distilled water only; an additional step (Step 3a) was added between Step 3 and Step 4, which consisted of heating the samples in a Ney STAR FIRE.TM. high performance programmable porcelain furnace (#949-14-002, J. M. Ney Company, Bloomfield, Conn.) at a particular furnace temperature for the respective metal substrate as follows: REXILLIUM.TM. III (Jeneric Industries, Inc., Wallingford, Conn.) and stainless steel 316 (3M Unitek) were heated at 1051.degree. C., RX NATURELLE.TM. (Jeneric Industries) was heated at 537.degree. C. and RX SP CG.TM. (Jeneric Industries) was heated at 1093.degree. C.; in Step 4, the organosilicon compound was applied by placing one drop of the A-174 solution on the surface of the metal disk with a plastic transfer pipet; and in Step 5, the samples were heated in the porcelain furnace described in Step 3a at 500.degree. C. for 10 minutes.

Printed by EAST

UserID: ETherkorn

Computer: WS08186

Date: 08/03/2000

Time: 10:02

	Type	L #	Hits	Search Text	DBs	Time Stamp
1	BRS	L1	194739	composite	USPAT	2000/08/03 09:34
2	BRS	L2	1415	210/198.2.ccls.	USPAT	2000/08/03 09:35
3	BRS	L3	147	1 and 2	USPAT	2000/08/03 09:35
4	BRS	L4	7694	resin NEAR7 silica	USPAT	2000/08/03 09:36
5	BRS	L5	23	3 and 4	USPAT	2000/08/03 09:45
6	BRS	L6	159153	chromatogra\$5	USPAT	2000/08/03 09:46
7	BRS	L7	1645	1 and 4	USPAT	2000/08/03 09:47
8	BRS	L8	268	6 and 7	USPAT	2000/08/03 09:47
9	BRS	L9	329	composite same (silica near7 resin)	USPAT	2000/08/03 09:49
10	BRS	L10	9918	1 and 6	USPAT	2000/08/03 09:50
11	BRS	L11	329	1 and 9	USPAT	2000/08/03 09:51
12	BRS	L12	70156	210/\$.ccls.	USPAT	2000/08/03 09:51
13	BRS	L13	3	9 and 12	USPAT	2000/08/03 09:53
14	BRS	L14	641	210/635.ccls.	USPAT	2000/08/03 09:54
15	BRS	L15	2	9 and 14	USPAT	2000/08/03 09:55
16	BRS	L16	1318	210/656.ccls.	USPAT	2000/08/03 09:55
17	BRS	L17	3	9 and 16	USPAT	2000/08/03 09:56
18	BRS	L18	873	210/502.1.ccls.	USPAT	2000/08/03 09:56
19	BRS	L19	2	18 and 9	USPAT	2000/08/03 09:57
20	BRS	L20	20372	pipet\$4	USPAT	2000/08/03 10:00
21	BRS	L21	4	9 and 20	USPAT	2000/08/03 10:01

	Comments	Error Definition	Errors
1			0
2			0
3			0
4			0
5			0
6			0
7			0
8			0
9			0
10			0
11			0
12			0
13			0
14			0
15			0
16			0
17			0
18			0
19			0
20			0
21			0